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ESTERIFICATION OF SERUM CHOLESTEROL

I. Serial Determinations in Health

Arnold Wagner, M.D.,* and Charles A. Poindexter, M.D.
Chicago, Ill.

WHEN serum is incubated at 37° C., the free cholesterol originally present gradually disappears, without change in the value for total cholesterol. The respective fractions are determined by digitonin precipitation before and after saponification. Sperry[1] has produced evidence that the disappearance of free cholesterol is explained by enzymatic esterification and has indicated the existence of a specific enzyme, cholesterol esterase. The enzymatic character of the reaction has been questioned recently by Swell and Treadwell.[2]

The work reported here was undertaken to test the constancy of the esterifying power of the serum from time to time in the same individual.

EXPERIMENTAL

Methods.—Estimations of the esterifying power of the serum were based upon the quantitative disappearance of free cholesterol from the serum during three days of incubation at 37.5° C. Blood was drawn from healthy young women sometimes in the fasting state but usually after breakfast, since at the time this work was done the effect of a meal was thought to be unimportant. Cholesterol determinations were done according to Sperry's modification[3] of the Schoenheimer and Sperry method.[4] Serum was incubated in the manner described by Sperry and Stoyanoff.[5]

RESULTS

The study included nineteen women ranging in age from 17 to 20 years, all of whom lived and ate together. Menstrual data are not available. From three to five determinations were done on the individuals in the series over periods of thirty-five to three hundred ninety-one days. The data for the entire group are summarized in Table I, which also details the serial results for two typical examples.

The range of total cholesterol concentration among the individuals studied was 112.6 to 245.2 mg. per cent. The mean total cholesterol concentration for all determinations was 181.7 ± 28.8 mg. per cent. The individual standard deviations ranged from 4.9 to 30.0 mg. per cent.

It is of interest that the esterifying power in the sera of nine middle-aged individuals was significantly less than that of the healthy group of young women. This difference is expressed by comparison of the mean combined: free ratios at one and three days of incubation. The values were, respectively, 3.90 ± 0.33 and 5.01 ± 0.84 for the older group, compared with 5.10 ± 0.75 and 7.47 ± 1.99 for the younger group.

From the Division of Cardiology, Department of Medicine, New York Postgraduate Medical School and the New York University Hospital, New York, N. Y.

Read, in part, at the Twenty-fourth Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., Nov. 2, 1951.

Received for publication, May 1, 1952.

*Now in the Department of Medicine, Northwestern University Medical School.

TABLE I. SERIAL DETERMINATIONS OF ESTERIFYING POWER OF SERUM IN HEALTH

SUBJECT	DAY OF OBSERVATION		CHOLESTEROL			
				RATIO: COMBINED:FREE		
			TOTAL, MG. PER CENT	INITIAL*	24 HOURS	72 HOURS
E. H.	0		216.4	2.86	4.56	7.66
	63		210.3	2.63	5.34	5.62
	146		186.7	2.67	4.74	8.02
	189		187.3	2.65	5.57	8.09
	352		191.1	2.67	5.28	8.38
		Mean	198.4	2.70	5.10	7.55
		S. D.†	±14.0	±0.09	±0.43	±1.11
L. H.	0		187.1	2.59	4.31	6.60
	64		199.6	2.47	5.28	6.03
	141		160.7	2.67	4.63	6.30
	190		169.5	2.80	5.32	8.02
		Mean	179.2	2.63	4.88	6.74
		S. D.	±17.5	±0.14	±0.50	±0.89
F. D.	0-379 days	Mean	193.9	2.36	4.35	5.42
	4 determinations	S. D.	±30.0	±0.18	±0.53	±1.48
C. E.	0-371 days	Mean	129.5	2.45	6.43	11.14
	5 determinations	S. D.	±11.5	±0.20	±1.15	±3.72
E. E.	0-239 days	Mean	203.5	2.48	3.97	5.08
	4 determinations	S. D.	±21.0	±0.12	±0.47	±0.99
G. F.	0-232 days	Mean	191.9	2.49	4.82	6.49
	4 determinations	S. D.	±19.1	±0.16	±0.17	±0.93
J. C.	0-223 days	Mean	150.6	2.38	5.40	7.94
	3 determinations	S. D.	±23.4	±0.23	±0.50	±0.61
S. C.	0-356 days	Mean	172.6	2.71	6.10	9.18
	4 determinations	S. D.	±5.3	±0.16	±0.40	±1.83
M. J.	0-140 days	Mean	188.1	2.56	5.08	7.31
	3 determinations	S. D.	±9.9	±0.06	±0.17	±1.64
O. K.	0-335 days	Mean	204.7	2.57	4.55	6.13
	5 determinations	S. D.	±12.5	±0.14	±0.41	±0.61
P. K.	0-356 days	Mean	221.2	2.63	4.89	6.61
	5 determinations	S. D.	±17.7	±0.08	±0.42	±1.09
P. L.	0-232 days	Mean	182.1	2.70	5.68	9.04
	4 determinations	S. D.	±23.5	±0.06	±0.67	±1.99
N. M.	0-126 days	Mean	190.2	2.69	5.19	7.30
	3 determinations	S. D.	±30.0	±0.04	±0.43	±1.01
R. R.	0-382 days	Mean	138.8	2.73	5.37	8.43
	5 determinations	S. D.	±20.8	±0.15	±0.48	±1.02
M. R.	0-334 days	Mean	190.1	2.57	5.02	8.01
	5 determinations	S. D.	±21.0	±0.24	±0.52	±1.21
E. R.	0-331 days	Mean	148.4	2.49	5.20	8.19
	5 determinations	S. D.	±7.2	±0.15	±0.70	±1.68
J. R.	0-155 days	Mean	179.0	2.57	5.08	7.82
	4 determinations	S. D.	±18.6	±0.23	±0.89	±0.49
D. S.	0-35 days	Mean	191.3	2.44	5.08	8.02
	3 determinations	S. D.	±4.9	±0.16	±0.48	±0.72
P. Y.	0-391 days	Mean	200.2	2.65	4.95	6.94
	4 determinations	S. D.	±10.7	±0.15	±0.44	±1.04

*Sperry's observation regarding the constancy of this ratio in health, 2.31 to 3.12,[1] is borne out by these data.

†S.D. $= \sqrt{\frac{\Sigma d^2}{n-1}}$

DISCUSSION

Inspection of data in Table I reveals that the esterifying power of the serum in any healthy individual is fairly constant, on repeated sampling, at twenty-four hours of incubation, and varies more widely at seventy-two hours as reflected by a larger standard deviation. That this individual variation is less than the variation among the individuals is proved by subjecting the data to analysis of variance.* At twenty-four hours of incubation the computed F value for the combined:free ratio was 3.73 compared to a table F value[7] of 2.27 at 99 per cent confidence limits. At seventy-two hours of incubation the respective F values were 3.14 and 2.76, establishing significance satisfactorily. It thus appears that the esterifying power of the serum fluctuates about a value that is "constitutional" for the individual, in health. These fluctuations appear to reflect metabolic alterations that can be appreciated in no other way. Thus, the initial determinations of the cholesterol partitions in all instances remained within the rigidly constricted norms.† Those determinations were in effect done on circulating blood. Only in the incubated aliquots did the degree of difference between sera become manifest. This difference did not seem to be related to the current concentration of cholesterol in the serum.

SUMMARY

The existence of a cholesterol-esterifying reaction in incubating serum is confirmed.

Evidence is presented that the variation of this function within individuals is less than the variation among individuals. The esterifying power of the serum of any healthy individual fluctuates within a range that is inherent in the individual constitution.

The reaction in a small group of middle-aged individuals was both less active and less variable than it was in the younger group.

REFERENCES

1. Sperry, W. M.: Cholesterol Esterase in Blood, J. Biol. Chem. **111**: 467, 1935.
2. Swell, L., and Treadwell, C. R.: Cholesterol Esterases: III. Occurrence and Characteristics of Cholesterol Esterase of Serum, J. Biol. Chem. **185**: 349, 1950.
3. Sperry, W. M.: A Micromethod for the Determination of Total and Free Cholesterol, Am. J. Clin. Path. **8**: (Tech. Supp.) **2**: 91, 1938.
4. Schoenheimer, R., and Sperry, W. M.: A Micromethod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**: 745, 1934.
5. Sperry, W. M., and Stoyanoff, V. A.: The Enzymatic Synthesis and Hydrolysis of Cholesterol Esters in Blood Serum, J. Biol. Chem. **121**: 101, 1937.
6. Pearson, F. A., and Bennett, R. R.: Statistical Methods, New York, 1942, John Wiley and Sons, p. 345ff.
7. Snedicor, G. W.: Statistical Methods Applied to Experiments in Agriculture and Biology, Ames, Iowa, 1940, Iowa State College Press, pp. 184-187.

*The method of Pearson and Bennett[6] was used. Variance is the average of the squared deviation about an arithmetic mean. Thus,

$$\frac{\Sigma\, x^2}{N-1} = \frac{\Sigma\, (X - AX)^2}{N-1} = \frac{\Sigma\, X^2 - (\Sigma\, X^2)}{\frac{N}{N-1}}$$

This formula was applied to the total number of determinations and to the variations among individuals. The difference between these two values is the contribution from variations inherent in the individual.

The F test of Pearson and Bennett[6] was then applied to contrast the variations among individuals with those within individuals. Thus,

$$F = \frac{\text{Variance among}}{\text{Variance within}}$$

†Sperry[1] observes that this ratio is constant in health, 2.31 to 3.12:1

ESTERIFICATION OF SERUM CHOLESTEROL

II. Influence of Phosphatide and Other Factors

Arnold Wagner, M.D., and Leonore Rogalski, B.S.
Chicago, Ill., and Evanston, Ill.

A PREVIOUS paper[1] described the variation of the in vitro esterification of free cholesterol in the blood serum of some healthy individuals. The present paper is concerned with further investigation of this reaction. The principal discovery has been that the addition to serum of a plant phosphatide mixture, containing a preponderance of lecithin, increases the amount of esterification.

METHODS

Freshly drawn human serum was incubated at 37° C. in sealed glass vials according to the technique described by Sperry and Stoyanoff.[2] Where necessary, serum was inactivated by heating to 56° C. for one hour. Reagents were added prior to incubation. With noted exceptions, the volume of such added reagents was adjusted throughout the experiments to a ratio of 1:5 with serum. We confirmed Sperry's finding that dilution of this order had no appreciable influence on the esterifying power of serum. Cholesterol determinations according to the Sperry modification[3] of the Schoenheimer-Sperry technique were performed on 1 ml. of serum or the serum-reagent mixtures.

The phosphatides of plant* origin supplied by the manufacturer were described as the alcohol-soluble extracts. Water emulsions of these phosphatides were freshly prepared in a Waring blendor prior to use. At the start of each experiment the phospholipid phosphorus content of these emulsions was determined on aliquots by the method of Zilversmit and Davis.[4] This method was also used for the phospholipid and acid-soluble phosphorus determinations on serum and serum-reagent mixtures. The acid-soluble phosphorus was determined on the supernate of the trichloroacetic acid precipitation. Total phosphorus determinations were done directly on the perchloric acid digest without trichloroacetic acid precipitation.

Emulsions of C. P. cholesterol† (melting point 149° C.) were prepared by the method of Cole, Clark, and Womach.[5]

Sperry[6] states that the disappearance of free cholesterol from the incubating serum without alteration of the total is explained by esterification. We adhere to his convention in expressing the degree of this esterification as the per cent decline in the free cholesterol originally present in the sample.

EXPERIMENTAL

Effect of Enzyme Concentration.—The amount of enzyme present in serum can be diminished quantitatively by serial dilution of active with inactivated aliquots of the same sample. The results of this procedure in four instances are shown in Fig. 1. The proportion

From the Department of Medicine, Northwestern University Medical School, Chicago, Ill., and the Evanston Hospital Association, Evanston, Ill.

Read, in part, at the Twenty-fourth Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., Nov. 2, 1951.

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Received for publication, May 1, 1952.

*The Glidden Company, Soya, Products Division, 5161 W. Moffet Avenue, Chicago, Ill. This material was described by the manufacturer as ". . . . . predominantly lecithin, but also having minor impurities of cephalin, inositol phosphatides, sugars, soybean oil, moisture and a petroleum ether insoluble impurity."

†Pfannstiehl Chemical Company, Waukegan, Ill.

of active to inactivated aliquot was varied from 0 to 100 per cent, and all samples were incubated for three days. The amount of free cholesterol esterified was directly proportional to the amount of active serum present in the mixture.

The totally inactivated aliquot of serum incubated for the unit time showed an *increase* of free cholesterol over the amount found in the similarly inactivated control aliquot that was not incubated. This unexplained phenomenon has been observed in many additional samples not shown here. This effect is the opposite of that found when unheated serum is used under the same conditions, as shown in Fig. 1.

Role of Extrinsic Cholesterol.—C. P. cholesterol (melting point 149° C.) was emulsified in a 1.48 per cent concentration and stabilized with 0.49 per cent sodium oleate. 0.1 ml. of

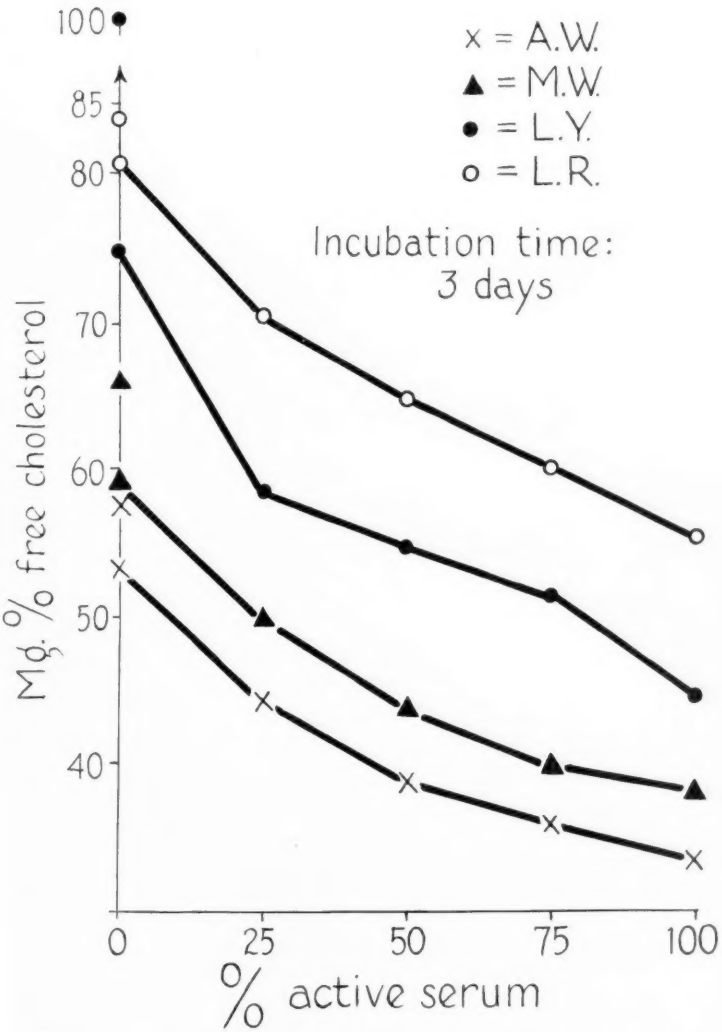

Fig. 1.—Serial dilutions of active with heat-inactivated aliquots of the same sample of serum. All samples on the zero ordinate were totally inactivated. The lower member of each pair was analyzed immediately after heat inactivation and within experimental error coincides with the value for the freshly drawn unheated serum. The upper member was incubated for the same length of time as all other samples in the figure. A significant trend toward *hydrolysis* of esterified cholesterol in samples thus treated is seen.

TABLE I. EFFECT OF THE ADDITION OF C.P. CHOLESTEROL TO INCUBATING SAMPLES OF SERUM

SUBJECT	REAGENT ADDED TO SERUM*	CHOLESTEROL IN SERUM						
		FREE		TOTAL		DISSOLVED FROM GLAZE (MG. %)	3 DAY DECLINE† IN FREE (MG. %)	NET DECLINE‡ IN FREE (MG. %)
		0 DAYS (MG. %)	3 DAYS (MG. %)	0 DAYS (MG. %)	3 DAYS (MG. %)			
M. D.	H_2O	57.4	36.3	212.0	208.0		21.1	21.1
	H_2O + cholesterol glaze	57.4	65.2	212.0	226.0	14.0	−7.8	6.2
	452 mg. % phosphatide	55.5	27.7	209.0	----		27.8	27.8
	452 + cholesterol glaze	55.5	69.1	209.0	225.0	16.0	−13.6	2.4
J. L. 1/14/50	H_2O	39.2	21.7	137.4	142.0		17.5	17.5
	H_2O + cholesterol glaze	39.2	41.9	137.4	152.0	14.6	−2.7	11.9
	420 mg. % phosphatide	36.7	19.9	147.5	142.0		16.8	16.8
	420 + cholesterol glaze	36.7	40.5	147.5	160.8	13.3	−3.8	9.5
1/28/50	H_2O	47.9	15.8	163.8	161.0		32.1	32.1
	H_2O + cholesterol glaze	47.9	54.7	163.8	162.8		−6.8	−6.8
	462 mg. % phosphatide	45.0	9.3	161.0	158.1		35.7	35.7
	462 + cholesterol glaze	45.0	54.7	161.0	163.6	2.6	−9.7	−7.1
W. K. 1/14/50	H_2O	65.0	41.8	221.0	----		23.2	23.2
	H_2O + cholesterol glaze	65.0	67.9	221.0	224.3	3.3	−2.9	0.4
	420 mg. % phosphatide	66.0	41.8	221.0	----		24.2	24.2
	420 + cholesterol glaze	66.0	73.6	221.0	232.0	11.0	−7.6	3.4
1/28/50	H_2O	60.9	31.4	208.0	206.9		29.5	29.5
	H_2O + cholesterol glaze	60.9	64.5	208.0	224.0	16.0	−3.6	12.4
	462 mg. % phosphatide	62.1	23.7	212.0	209.5		38.4	38.4
	462 + cholesterol glaze	62.1	73.6	212.0	221.0	9.0	−11.5	−2.5
R. S.	H_2O	50.7	35.2	184.0	184.0		15.5	15.5
	H_2O + cholesterol glaze	50.7	57.0	184.0	190.3	6.3	−6.3	0
	452 mg. % phosphatide	46.2	19.8	175.0	175.0		26.4	26.4
	452 + cholesterol glaze	46.2	61.3	175.0	200.8	25.8	−15.1	10.7
E. S.	H_2O	43.3	25.6	164.0	164.0		17.7	17.7
	H_2O + cholesterol glaze	43.3	65.6	164.0	172.6	8.6	−22.3	−13.7
	325 mg. % phosphatide	45.5	14.8	173.0	173.0		30.7	30.7
	325 + cholesterol glaze	45.5	60.5	173.0	183.0	10.0	−15.0	−5.0
D. T.	H_2O	28.3	17.2	111.3	106.7		11.1	11.1
	H_2O + cholesterol glaze	28.3	46.0	111.3	118.5	7.2	−17.7	−10.5
	325 mg. % phosphatide	28.3	7.8	108.5	111.4		20.5	20.5
	325 + cholesterol glaze	28.3	47.4	108.5	118.9	10.4	−19.1	−8.7
S. L.	H_2O	52.5	28.9	184.0	183.5		23.6	23.6
	H_2O + cholesterol glaze	52.5	49.5	184.0	197.0	13.0	3.0	16.0
	325 mg. % phosphatide	54.5	15.8	187.0	181.0		38.7	38.7
	325 + cholesterol glaze	54.5	72.1	187.0	199.0	12.0	−17.6	−5.6

TABLE I—CONT'D

SUBJECT	REAGENT ADDED TO SERUM*	CHOLESTEROL IN SERUM						
		FREE		TOTAL		DISSOLVED FROM GLAZE (MG. %)	3 DAY DECLINE† IN FREE (MG. %)	NET DECLINE‡ IN FREE (MG. %)
		0 DAYS (MG. %)	3 DAYS (MG. %)	0 DAYS (MG. %)	3 DAYS (MG. %)			
B. G.	H_2O	37.5	20.3	150.2	149.6		17.2	17.2
	H_2O + cholesterol glaze	37.5	46.1	150.2	164.9	14.7	–8.6	6.1
	452 mg. % phosphatide	39.5	13.5	149.0	152.3		26.0	26.0
	452 + cholesterol glaze	39.5	50.1	149.0	171.8	22.8	–10.6	12.2
J. A.	H_2O	49.1	28.3	183.8	183.5		20.8	20.8
1/14/50	H_2O + cholesterol glaze	49.1	54.1	183.8	188.8	5.0	–5.0	0
	420 mg. % phosphatide	49.1	32.4	182.8	183.5		16.7	16.7
	420 + cholesterol glaze	49.1	58.2	182.8	192.0	9.2	–9.1	0.1
1/28/50	H_2O	48.3	22.6	178.0	182.6		25.7	25.7
	H_2O + cholesterol glaze	48.3	62.3	178.0	192.5	14.5	–14.0	0.5
	462 mg. % phosphatide	48.9	11.0	178.3	182.6		37.9	37.9
	462 + cholesterol glaze	48.9	54.4	178.3	192.5	14.5	–5.5	9.0

*Serum: reagent mixture was in 5:1 ratio.

†Where negative signs appear, apparent hydrolysis occurred.

‡The net decline equals the original free cholesterol plus dissolved cholesterol minus the value for free cholesterol after seventy-two hours of incubation. Unlike those in the preceding column, a negative sign in this column indicates some actual hydrolysis has occurred.

this emulsion was added per 1.0 ml. of serum. In another preparation cholesterol was melted as a glaze on the inside of the incubating vial. Two cubic centimeters of serum were added to the vial. All samples of both preparations were incubated for seventy-two hours, and filtered through sintered glass funnels of fine porosity prior to analysis.

Where emulsified cholesterol was added to two sera, there was no increase in the amount of cholesterol esterified, over the control determinations on serum alone. This confirms Sperry's experience with similar preparations. Where cholesterol was dissolved from the glaze, in another twelve sera from nine subjects, less than control amounts were esterified in all instances. In three of the twelve sera, hydrolysis of naturally occurring esterified cholesterol was found (Table I, column 7). It is clear that extrinsic cholesterol not only is not esterified, but actually inhibits the esterification of naturally-occurring free cholesterol in the serum to which it is added.

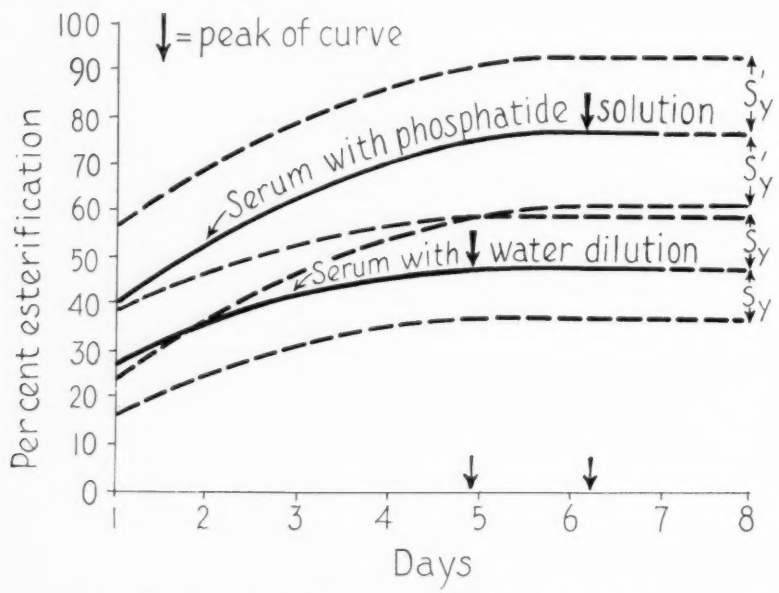

Fig. 2.—Influence of phosphatide on speed and equilibrium of the reaction. (*a*) Second-degree parabolic curve derived from 138 determinations (first to seventh days of incubation) on 22 sera. Sixty-eight per cent of all determinations should fall within the range of $Y_c \pm S_y$, where Y_c is the computed per cent of esterification and the S_y is the standard error of estimate. (*b*) A similarly derived curve based upon 131 determinations on 25 sera to which varying quantities of phosphatide were added.

The Phosphatide Effect.—Serum was incubated with and without added phosphatide for a period of seven days. Fig. 2 consolidates the data on twenty-two subjects. Detailed data on nine subjects are given in Table II. The speed of the reaction is increased, as indicated by the relative decrements of free cholesterol between control and experimental results at the end of two days of incubation.* The sample containing phosphatide reaches a new equilibrium point somewhat later than that of the control. We have collected six normals in whom the reaction has gone to completion, i.e., all free cholesterol esterified in the presence of phosphatide.

*In two subjects, the reaction was inhibited. One of these (W. B.) was an advanced case of atherosclerosis with hypertensive heart failure. The other (M. P.) showed inhibition of the reaction twenty-two days after myocardial infarction when phosphatide dispersion in stock concentration of 2,000 mg. per cent was used, but typical acceleration six months after infarction when phosphatide dispersion in stock concentration of 1,000 mg. per cent was used. Dilution factors as shown made the absolute phosphatide concentration in both studies of M. P.'s serum approximately equal. While no conclusion can be drawn, it appears that the significant variable in both of these cases may have been disease.

The relationship of phosphatide to the esterifying reaction is altered in the presence of extrinsic cholesterol, as shown in Table I, column 7. In no instance was esterification increased. All were inhibited and in five of the twelve sera hydrolysis occurred.

Dependance of Phosphatide Effect Upon Phosphatide Concentration.—A typical experiment is demonstrated in Fig. 3. The esterification of cholesterol is enhanced in direct proportion as the quantity of available phosphatide is increased. This is true only in the relatively lower range of phosphatide concentration; in the higher range the effect is lost. A typical instance of diminishing effect with increasing concentration is detailed in Table III.

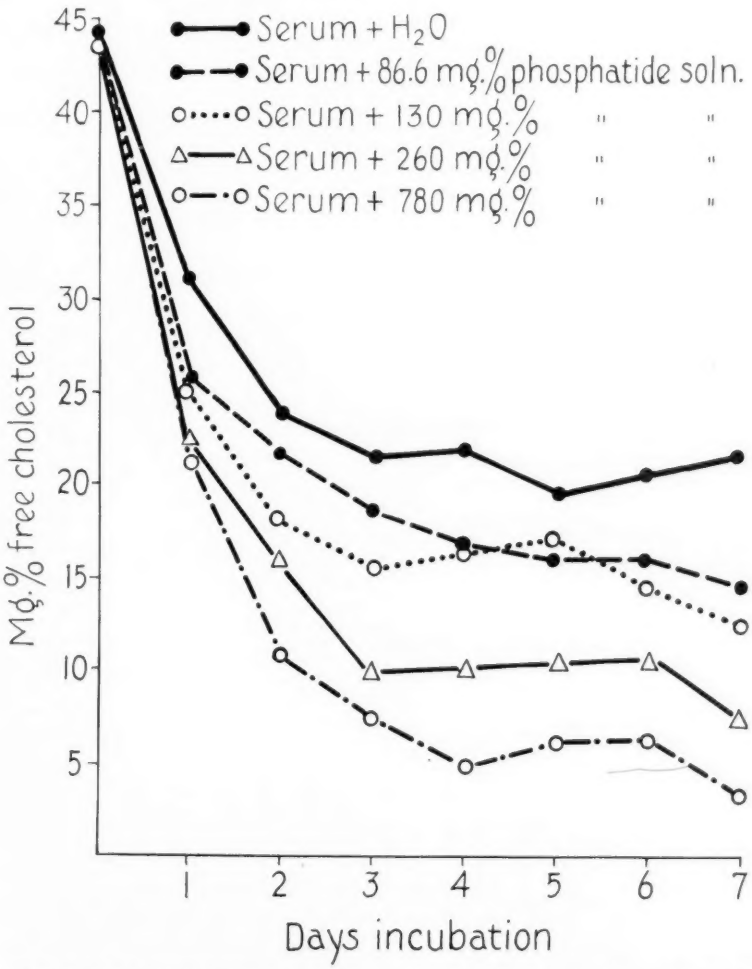

Fig. 3.—The relationship between concentration of phosphatide and the degree to which the free serum cholesterol is esterified. All five aliquots derived from a single sample of serum. The figures on phosphatide concentration apply before addition to serum in a 1:5 ratio.

Relation of Phosphatide Effect to Esterifying Enzyme.—One cubic centimeter of heat-inactivated serum was incubated for varying periods with 0.2 c.c. of approximately 2 per cent phosphatide dispersion. Five subjects were studied. The results were uniformly negative throughout, in that no esterification occurred. In three of the five subjects, the tendency was rather toward an increase of hydrolysis by comparison with the inactivated control

TABLE II. EFFECT OF SOYBEAN PHOSPHATIDE UPON IN VITRO ESTERIFICATION

SUBJECT	REAGENT ADDED TO SERUM*	BEFORE INCUBATION		AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS
		CHOLESTEROL					
		TOTAL (MG. %)	FREE (MG. %)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)
	Normal Sera						
L. R.	H_2O	246.5	64.3	23.4	33.6	32.2	35.6
	1608 mg. % phosphatide†	248.3	64.3	23.4	41.8	48.4	58.1
B. R.	H_2O	183.5	51.5	28.2	48.7	41.5	47.1
	1745 mg. % phosphatide	189.0	52.5	53.3	58.1	68.2	74.1
M. P.	*Others*						
4-10-50	H_2O	177.2	51.1	19.4	32.7	30.1	37.0
	2000 mg. % phosphatide	179.8	52.0	9.6	10.0	19.5	25.8
10-3-50	H_2O‡	201.5	54.1	26.4	30.5	27.2	38.0
	1000 mg. % phosphatide‡	202.4	54.1	23.3	39.9	47.3	53.6
C. M.	H_2O	173.8	47.0	22.4	33.2	37.9	42.1
	2000 mg. % phosphatide	174.0	50.0	35.8	62.8	75.0	87.7
M. L.	H_2O	163.5	44.9	22.9	34.8	30.5	38.1
	2000 mg. % phosphatide	166.7	44.9	25.2	49.5	41.9	66.0
W. B.	H_2O‡	154.7	44.5	26.3	24.7	39.3	36.9
	1560 mg. % phosphatide‡	158.8	46.6	29.2	13.1	47.2	52.5
M. R. W.	H_2O	135.0	38.0	30.0	51.3	45.8	48.1
	1601 mg. % phosphatide	132.5	35.8	28.5	53.1	51.2	57.6
B. W.	H_2O	149.5	40.7	28.8	45.0	43.0	46.2
	1608 mg. % phosphatide	151.5	41.8	43.1	58.6	71.0	71.3
A. W.	H_2O	155.8	42.0	28.1	47.8	51.6	52.4
	1428 mg. % phosphatide	158.4	43.5	38.8	73.1	89.5	100.0

*Serum: reagent mixture was in 5.1 ratio.

†The concentration of phosphatide as expressed in this table was derived by multiplying the determined phosphate values by 25.

‡Serum: reagent mixture was in 2.5:1 ratio.

TABLE III. EFFECT OF PHOSPHATIDE CONCENTRATION

SUBJECT (SINGLE SAMPLE)	REAGENT ADDED TO SERUM (1:5)	BEFORE INCUBATION		AFTER 1 DAY	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 5 DAYS	AFTER 6 DAYS	AFTER 7 DAYS
		CHOLESTEROL							
		TOTAL (MG. %)	FREE (MG. %)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)	ESTERIFICATION (%)
B. R.	H_2O	191.3	58.7	35.2	50.9	47.0	58.4	45.2	46.5
	288 mg. % phosphatide	182.2	60.0	48.2	75.9	67.1	57.3	46.3	62.7
	864 mg. % phosphatide	193.8	59.0	41.2	94.3	87.6	89.8	100.0	94.6
	1440 mg. % phosphatide	195.3	61.0	49.8	96.4	82.5	86.6	94.9	94.3
	2880 mg. % phosphatide	186.7	61.0	38.0	48.9	57.1	55.1	56.3	47.0

samples not containing phosphatide. The active control aliquots displayed the usual degree of activity. The potentiating action of the phosphatide mixture is therefore not independent of the esterifying enzyme.

Further Exploration of the Phosphatide Effect.—Suitable experiments were done to discover whether the effect of phosphatide could be identified in some of its fractions or in other materials having similar properties. However, the effect could not be reproduced with choline, glycerol, sodium oleate, or sodium stearate, nor with Tween 80, saponin, or sodium choleate, nor with adenosine triphosphate, sodium pyrophosphate, or cozymase. The phosphatide effect was not inhibited by sodium fluoride.

Role of Lecithin.—Because of the lability of the fatty acid side chains in lecithin,[7-9] the relative abundance of this material in the blood stream[10, 11] and the parallel fluctuations of total cholesterol and phospholipid in health,[11] it was thought that lecithin could be the donor of the fatty acids in the in vitro esterification of cholesterol. This surmise has been anticipated by others,[6, 12, 13] but with little supporting evidence. If lecithin supplies the acyl radical, proof should be available in a demonstrable increase in glycerocholine phosphate as a product of the reaction, or in a decrease of measureable phospholipid at the equilibrium point of the reaction, or possibly in a demonstration of lysolecithin at some point in the reaction. All of these possibilities were carefully developed in suitably controlled experiments. While it was possible to show clearly that no lysolecithin is formed during incubation of serum, the experiments bearing upon the other two possibilities were inconclusive owing to technical difficulties detailed below.

DISCUSSION

In the absence of a standard measure of the reaction in question, our information must derive from such indirect evidence as has been presented here. It is not reasonable to expect an early definition of unit esterase activity until the existence of the disputed enzyme is proved by isolation and the one or more reactions leading to net esterification of cholesterol are identified. The measurement of serum lecithin before and after incubation might have identified one of the reactions involved, but failed for technical cause. It was found that spontaneous oxidation of this phosphatide occurred during incubation, independently of the esterification of cholesterol. The measurement of glycerocholine phosphate as a product of the same reaction proved inadequate for blood samples of reasonable size.

In the experiments involving the addition of C. P. cholesterol to serum, the fact that the added cholesterol was able to pass through a sintered glass funnel of fine porosity with the serum indicates that the dispersion was fine enough to favor biochemical activity. The fact that this cholesterol was not esterified suggests a biochemical difference between extrinsic and naturally occurring serum cholesterol.

The discovery that a plant phosphatide mixture is capable of increasing the net esterification of serum cholesterol in vitro appears to provide a useful tool for further investigation of serum lipid metabolism. Our work has established thus far that the phosphatide effect depends upon the coexistence of a heat-labile component of serum, that it is probably independent of emulsifying characteristics and that within limits it is quantitative. Our findings are of particular interest in that they provide a direct biochemical link between two important constituents of blood; the cholesterol fractions on one hand and phosphatides on the other.

SUMMARY

The work of Sperry regarding the existence of a cholesterol-esterifying principle in blood has been confirmed and extended. His observations relative to the inert role of extrinsic cholesterol in this system have been in part corroborated. We have further discovered that when normal serum is incubated in contact with a glaze of melted C. P. cholesterol, the esterification which would otherwise occur in the serum is either inhibited or reversed.

We have found that the addition to healthy serum, before incubation, of a mixture of soybean phosphatides regularly results in an increase of cholesterol esterification. The speed of the reaction is increased and a new equilibrium established.

The phosphatide effect depends upon the coexistence of a heat-labile component of serum. It is independent of emulsifying characteristics. Within limits it is quantitative.

The phosphatide effect is lost in the presence of extrinsic cholesterol.

The authors wish to express their appreciation to Professor Byron Riegel whose advice helped to govern the course of the investigation. Mr. Alexander Farlinger assisted with the statistical analysis.

REFERENCES

1. Wagner, A., and Poindexter, C. A.: Esterification of Serum Cholesterol, I. Serial Determinations in Health, J. Lab. & Clin. Med. **40**: 321, 1952 (this issue).
2. Sperry, W. M., and Stoyanoff, V. A.: The Enzymatic Synthesis and Hydrolysis of Cholesterol Esters in Blood Serum, J. Biol. Chem. **121**: 101, 1937.
3. Sperry, W. M.: A Micromethod for the Determination of Total and Free Cholesterol, Am. J. Clin. Path. 8(Tech. Supp.) **2**: 91, 1938.
4. Zilversmit, D. B., and Davis, A. K.: Microdeterminations of Plasma Phospholipids by Trichloroacetic Acid Precipitation, J. Lab. & Clin. Med. **35**: 155, 1950.
5. Cole, W. H., Clark, H., and Womach, M. A.: Preparation of Cholesterol Emulsion for Experimental Intravenous Administration, J. Lab. & Clin. Med. **26**: 1679, 1941.
6. Sperry, W. M.: Cholesterol Esterase in Blood, J. Biol. Chem. **111**: 467, 1935.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry Interpretations, Volume I, ed. 2, Baltimore, 1946, Williams and Wilkins Company, pp. 380-386.
8. Sinclair, R. G.: The Metabolism of the Phospholipids. VIII. The Passage of Elaidic Acid into Tissue Phospholipids. Evidence of the Intermediary Role of Liver Phospholipid in Fat Metabolism, J. Biol. Chem. **111**: 515, 1935.
9. Sinclair, R. G.: Blood Phospholipid as a Transport Mechanism, J. Biol. Chem. **115**: 211, 1936.
10. Page, I. H., Kirk, E., Lewis, W. H., Thompson, W. R., and Van Slyke, D. D.: Plasma Lipids of Normal Men at Different Ages, J. Biol. Chem. **111**: 613, 1935.
11. Kornerup, V.: Concentrations of Cholesterol, Total Fat and Phospholipid in Serum of Normal Man, Arch. Int. Med. **85**: 398, 1950.
12. Berguhen, B.: Experimentalle Untersuchungen Über die Spontanen Veranderungen des Blutes *in Vitro*, Acta. Patho. et Microbiol. Scandinav., Supplement **39**: 1938.
13. LeBreton, E., and Pantaleon, Jean: Mise en evidence d'une lecithase dans le serum in le plasma de mammiferes, Compt. rend. soc. de biol. **138**: 36, 1944.

ESTERIFICATION OF SERUM CHOLESTEROL

III. In Hypercholesterolemic Rabbits

Arnold Wagner, M.D.,* and Leonore Rogalski, B.S.
Chicago, Ill.

IT HAS been shown previously[1, 2] that when C.P. cholesterol is added to serum in vitro, it is inert or inhibitory with respect to the cholesterol-esterifying reaction that otherwise occurs upon incubation of the sample. The present paper is concerned with the comparative behavior of cholesterol that has been added to rabbit serum in vivo, via the normal route across the alimentary interface. When such hypercholesterolemic sera are incubated, the results in less than half are consistent with those following in vitro additions of cholesterol to serum. There is considerable individual variation and some animals show an *increase* in the esterifying power of the serum.

MATERIALS AND METHODS

Fourteen healthy male pigmented rabbits, ranging in age from four to six months and in weight from 2.18 to 4.27 kilograms, comprised the experimental material. The esterifying power of the serum was estimated as in our previous work[3] initially and at the conclusion of the feeding period. Briefly, the free, total, and combined (by difference) cholesterol of the sample were determined by the method of Sperry[4] before and after seventy-two hours of incubation at 37° C. Hypercholesterolemic samples were diluted in the extraction as needed to bring the cholesterol content within accurate experimental range. The esterifying power of the serum was expressed by the altered combined:free ratio in the sample after incubation. The experimental diet, taken ad libitum, contained 0.5 Gm. of cholesterol per hundred grams of ground Purina rabbit pellets. The cholesterol was dissolved in an ether and olive oil mixture, mixed with the ground pellets and the ether allowed to evaporate. Each animal consumed an average of 200 Gm. per day. The diet, supplemented by carrots, was maintained for 108 days.

RESULTS

The results are given in Table I. In 6 of the 14 cases (rabbits Nos. 56, 58, 63, 64, 65, and 67, column No. 7) less cholesterol was esterified after prolonged cholesterol feeding. In 2 additional cases (rabbits Nos. 34 and 60) this reversal of the usual result amounted to hydrolysis of esterified cholesterol. In the remaining 6 cases (rabbits Nos. 51, 54, 57, 59, 62, and 66) more cholesterol was esterified after the feeding period.

From the Department of Medicine, Northwestern University Medical School, Chicago, Ill.

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*Present address: 636 Church Street, Evanston, Ill.

TABLE I. PARTITION OF SERUM CHOLESTEROL IN THE HYPERCHOLESTEREMIC RABBIT

RABBIT NO.	TOTAL* 0 DAYS (MG. %)	TOTAL* 108 DAYS (MG. %)	RATIO COMBINED:FREE† 0 DAYS	RATIO COMBINED:FREE† 108 DAYS	ABSOLUTE INCREASE OF FREE AT 108 DAYS (MG. %)	DECLINE OF FREE UPON 72 HRS. INCUBATION 0 DAYS (MG. %)	DECLINE OF FREE UPON 72 HRS. INCUBATION 108 DAYS (MG. %)
34	72.9	764.0	1.40	2.25	224.7	16.6	−6.0‡
51	125.6	1325.0	1.81	2.54	331.0	11.3	25.0
54	110.5	766.0	2.54	2.43	187.9	6.2	13.0
56	84.5	715.5	2.08	2.91	155.1	7.6	4.6
57	61.0	885.0	2.21	2.33	237.6	10.3	40.9
58	43.6	322.5	1.62	3.02	66.0	10.2	7.7
59	40.6	681.5	1.96	2.88	167.8	9.5	12.0
60	61.2	657.2	2.09	2.88	151.3	14.8	−4.2
62	28.5	803.5	0.92	3.17	180.2	9.3	16.0
63	53.0	803.5	1.71	2.79	194.2	13.1	8.5
64	122.0	879.5	2.06	2.51	210.6	23.3	20.0
65	78.0	776.5	1.94	2.71	182.2	13.9	1.5
66	77.8	801.0	1.99	2.47	195.4	19.5	23.5
67	62.2	670.0	1.51	2.69	153.4	18.1	9.5
Mean	73.0	775.4	1.85	2.68			
σ§			±0.40	±0.27			

*Our results for a total of 76 untreated healthy rabbits including the present 14 were as follows: Total serum cholesterol 60.7 mg. per cent ± 22.2. Initial ratio of combined: free 1.46 ± 0.55. The mean free cholesterol esterified in three days was 13.3 mg. per cent ± 5.9.

†This ratio of combined:free was determined before incubation of the samples; i.e., in the control specimens.

‡The negative sign denotes an increase of free cholesterol instead of the usual decrease.

§$\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}$

DISCUSSION

Examination of the data reveals that the esterification performance in the hypercholesterolemic sera cannot be correlated with the initial or final cholesterol concentrations in the respective animals, the initial or final combined: free ratios, or the absolute increments of free cholesterol during the feeding period. For example, rabbits Nos. 59 and 60 are comparable in the initial values for total cholesterol, in the heights to which the total values rose after feeding, and in the ratios of combined:free cholesterol finally, in the hypercholesterolemic sera. Despite this superficial similarity, after the feeding period, rabbit serum No. 59 showed a loss of 12.0 mg. per cent of free cholesterol on incubation while rabbit serum No. 60 showed a gain of 4.2 mg. per cent of free cholesterol. This unpredictable performance indicates that the esterification involves more than mass action. The sera of all the animals before feeding consistently showed esterifying ability only, yet after feeding this characteristic varied from potentiation, through inhibition, to reversal or hydrolysis.

Why the induction of hypercholesterolemia should so inconsistently affect the esterifying potential of the serum is not known. We have found previously in human sera that the addition of cholesterol in vitro prior to incubation inhibits or reverses the processes which otherwise result in net esterification.[2] The possibility was considered, then, that the added cholesterol was biologically different from that which occurred naturally in the serum; i.e., perhaps lacking in essential linkages that only the living organism could provide. Similarly, in the present instance, the differences may reflect the variable ability of the

animal to provide other constituents of the reactive interface. In this connection the work of Dubach and Hill[5] and others[6, 7] who demonstrated alterations of albumin:globulin ratios during induced hypercholesterolemia in rabbits, may be pertinent.

It is seen in the table that the ratio of combined:free cholesterol rose significantly in most animals during the feeding period, indicating a disproportionate increase in esterified cholesterol. This finding confirms that of Dubach and Hill[5] and counters the observation of Hueper.[8] Further, all of the animals tended to approach the same maximum ratio at the conclusion of the feeding period, despite the relatively large variability of ratio before feeding. This was true regardless of whatever effect the hypercholesterolemia had upon the esterifying ability of the serum. It suggests that the synthesizing cholesterol esterase present in serum is not the primary factor in the regulation of the serum cholesterol partition in hypercholesterolemic rabbits.

SUMMARY

1. The esterifying power of the serum of 14 rabbits has been studied before and after the induction of hypercholesterolemia. All the animals showed a fairly uniform cholesterol esterifying ability in the serum before cholesterol feeding. The induction of hypercholesterolemia changed this characteristic quantitatively in an unpredictable fashion, and sometimes qualitatively, producing reversal or hydrolysis. The results could not be correlated with initial or final cholesterol concentrations, the initial or final combined:free ratios, or the absolute increments of free cholesterol during the feeding period.

2. It has been confirmed that during cholesterol feeding the esterified cholesterol reaches disproportionately greater heights in vivo than does the free fraction. Lack of correlation between this finding and the alteration in the esterifying ability of the serum suggest that the synthesizing cholestrol esterase present in serum is not primary in the regulation of the serum cholesterol partition of hypercholesterolemic rabbits.

REFERENCES

1. **Sperry, W. M.:** Cholesterol Esterase in Blood, J. Biol. Chem. **111:** 467, 1935.
2. **Wagner, A., and Rogalski, L.:** Esterification of Serum Cholesterol. II. Influence of Phosphatide and Other Factors, J. LAB. & CLIN. MED. **40:** 324, 1952 (this issue).
3. **Wagner, A., and Poindexter, C. A.:** Esterification of Serum Cholesterol. I. Serial Determinations in Health, J. LAB. & CLIN. MED. **40:** 321, 1952 (this issue).
4. **Sperry, W. M.:** A Micromethod for the Determination of Total and Free Cholesterol, Am. J. Clin. Path., 8 (Tech. Supp.) **2:** 91, 1938.
5. **Dubach, R., and Hill, R. M.:** The Effect of a Sustained Hypercholesterolemia on the Lipids and Proteins in the Plasma of the Rabbit, J. Biol. Chem. **165:** 521, 1946.
6. **Mauriac, P., Servanti, L., and Demenier, G.:** Variations du rapport serine-globuline dans le sang de lapins hypercholesteremiques, Compt. rend. soc. de biol. **115:** 177, 1934.
7. **Schwartz, H., and Lichtenberg, H. H.:** Relationship of the Blood Cholesterol to the Hemoglobin and Serum Protein, J. Biol. Chem. **121:** 315, 1937.
8. **Hueper, W. C.:** Arteriosclerosis. The Anoxemia Theory, Arch. Path. **39:** 192, 1945.

FEMORAL ARTERIOVENOUS SUGAR DIFFERENCES IN FASTING HUMAN BEINGS

DAVID M. BELL, M.D.
DURHAM, N. C.

THE purpose of this paper is to report the range of the femoral A-V glucose differences in a series of fasting subjects with normal carbohydrate metabolism.

METHOD

Studies were conducted upon hospital patients on routine hospital diets and upon seven normal students. They had no metabolic disorders, and the nutritional state was good. All observations were made in the morning, and the subjects had received no food since the previous evening. Using local procaine anesthesia, needles were inserted into the femoral artery and vein of the same leg, and blood samples were taken simultaneously.

The titration method of Somogyi[5, 6] with barium hydroxide and zinc sulfate precipitation of protein was used in all glucose determinations. In evaluating the method and checking it from time to time during the course of the study, a sample of blood was drawn into one syringe, and it was then distributed to a varying number of tubes as replicate samples, as indicated in the left column of Table I. The tubes were prepared by evaporating in them 1 ml. of a 3 per cent solution of sodium fluoride. A dilute solution of heparin (12½ mg. per milliliter) was used in the syringes as an anticoagulant. The mean concentration in milligrams per 100 ml. is listed in the center column, and indicates the range of values studied. The right column presents the standard deviations from the mean in each group. The largest standard deviation was ±0.71 milligrams per 100 ml. From these results, the average or mean standard deviation as calculated from the pooled variance is ±0.47 milligrams per 100 ml.

In this study and other work, 630 blood samples were analyzed for glucose concentration. Filtrates from these bloods were run in duplicate. The standard deviation of the variations obtained in these 630 pairs of filtrates was ±0.35 milligrams per 100 ml. of glucose as compared to the standard deviation of ±0.47 milligrams per 100 ml. for whole blood.

Blood oxygen was determined by the method of Hickam and Frayser.[11]

TABLE I. EVALUATION OF THE TITRATION METHOD OF SOMOGYI FOR THE DETERMINATION OF BLOOD SUGAR

NUMBER OF REPLICATE BLOOD SAMPLES	MEAN GLUCOSE CONCENTRATION ± STANDARD DEVIATION MG. PER 100 ML.
4	78.9 ± 0.36
4	83.6 ± 0.30
5	98.6 ± 0.42
5	123.1 ± 0.69
2	69.9 ± 0.50
2	74.9 ± 0.71
3	77.4 ± 0.33
3	85.7 ± 0.33

From the Department of Medicine, School of Medicine, Duke University.

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TABLE II. DISTRIBUTION OF FEMORAL ARTERIOVENOUS GLUCOSE DIFFERENCES. 80 STUDIES IN 60 SUBJECTS AT REST IN FASTING STATE

NUMBER OF STUDIES	ARTERIOVENOUS GLUCOSE DIFFERENCE (MG. PER 100 ML.)	MEAN ARTERIAL GLUCOSE VALUE ± STANDARD DEVIATION
4	−1.3 – −1	87.6 ± 5.5
10	−1 – 0	87.1 ± 10.8
11	0.1 – 0.9	81.1 ± 7.5
11	1 – 1.9	86.1 ± 9.8
12	2 – 2.9	88.0 ± 4.4
15	3 – 3.9	87.8 ± 7.0
9	4 – 4.9	85.9 ± 11.5
4	5 – 5.9	93.4 ± 5.4
4	6 – 6.7	91.8 ± 9.0

Mean arteriovenous difference ± S.D. = 2.20 ± 2.17 mg.%
Mean arteriovenous difference ± S.E. = 2.20 ± 0.24 mg.%
$t = 9.1$ $p < 0.01$

RESULTS

The femoral A-V glucose difference was measured eighty times in sixty subjects. The average difference was 2.2 milligrams per 100 ml. Table II presents the frequency distribution of the values obtained, and also indicates the approximate range of arterial glucose concentration for each class interval. This demonstrates that the data were collected from subjects with normal blood sugar concentrations. The actual range of A-V differences was −1.3 milligrams per 100 ml. to +6.7 milligrams per 100 ml.

Twenty-three subjects had repeat resting blood samples drawn at intervals varying from two to twenty-five minutes. In eight of these, blood for oxygen determination was also obtained. Table III contains the data where both glucose and oxygen studies were done, and Table IV contains the data where only glucose studies were performed. There appears to be no correlation between the oxygen uptake and the glucose uptake. Wide A-V oxygen differences, which in

TABLE III. GLUCOSE AND OXYGEN ARTERIOVENOUS DIFFERENCES REPEATED AT VARYING INTERVALS IN 8 HOSPITAL PATIENTS

TIME OF BLOOD SAMPLING	GLUCOSE MG. PER 100 ML.				OXYGEN VOL. %
	ARTERY	VEIN	A-V	VARIATION A-V	A-V
0 min.	80.8	76.8	4.0		4.8
15 min.	81.2	76.9	4.3	0.3	
0 min.	78.5	75.8	2.7		3.6
25 min.	79.4	76.3	3.1	0.4	3.2
0 min.	92.9	91.1	1.8		3.8
25 min.	93.3	91.3	2.0	0.2	5.7
0 min.	70.9	70.8	0.1		2.1
25 min.	71.1	70.9	0.2	0.1	2.3
0 min.	89.4	85.7	3.7		4.2
15 min.	90.1	87.3	2.8	0.9	
0 min.	83.5	83.9	−0.4		8.4
5 min.	83.6	83.6	.0	0.4	10.4
0 min.	90.8	89.5	1.3		7.9
2 min.	91.2	89.2	2.0	0.7	11.6
0 min.	103.0	101.7	1.3		4.2
2 min.	101.7	100.4	1.3	0.0	4.5

TABLE IV. FEMORAL GLUCOSE ARTERIOVENOUS DIFFERENCES REPEATED AT REST DURING VARYING PERIODS OF TIME 8 HOSPITAL PATIENTS, 7 NORMAL STUDENTS

TIME OF BLOOD SAMPLING	GLUCOSE MG. PER 100 ML.			
	ARTERY	VEIN	A-V	VARIATION A-V
0 min.	77.0	73.7	3.3	
10 min.	76.8	75.8	1.0	2.3
0 min.	77.9	75.5	2.4	
10 min.	78.5	75.7	2.8	0.4
0 min.	99.4	94.3	5.1	
10 min.	99.4	93.4	6.0	0.9
0 min.	78.4	78.1	0.3	
10 min.	82.0	77.1	4.9	4.6
0 min.	79.2	80.4	−1.2	
10 min.	82.2	79.2	3.0	4.2
0 min.	90.6	90.4	0.2	
10 min.	88.8	90.1	−1.3	1.5
0 min.	74.6	75.2	−0.6	
5 min.	72.2	72.2	0.0	0.6
0 min.	107.2	108.0	−0.8	
5 min.	107.5	107.8	−0.5	0.5
0 min.	87.3	86.6	0.7	
5 min.	87.6	85.0	2.6	1.9
0 min.	76.7	78.0	−1.3	
5 min.	77.7	76.5	1.2	2.5
0 min.	76.4	75.4	1.0	
5 min.	77.0	74.6	2.4	1.4
0 min.	86.4	83.8	2.6	
5 min.	87.3	84.9	2.4	0.2
0 min.	79.8	76.3	3.5	
5 min.	79.6	76.0	3.6	0.1
0 min.	78.6	76.4	2.2	
5 min.	77.7	77.1	0.6	1.6
0 min.	85.2	80.6	4.6	
10 min.	83.6	80.4	3.2	1.4

Mean change in arteriovenous difference ± standard deviation = 1.2 ± 1.2 milligrams per 100 ml.

Mean change in arterial glucose concentration ± standard deviation = 0.9 ± 0.9 milligrams per 100 ml.

Mean change in venous glucose concentration ± standard deviation = 0.8 ± 0.8 milligrams per 100 ml.

The mean values listed above apply to the combined data of Table III and Table IV.

the resting state are suggestive of slow blood flow, may be associated with negligible A-V glucose differences. The arterial and venous glucose concentrations and A-V glucose differences were found to remain remarkably constant during the time intervals observed. The mean variation ± the standard deviation of the mean for the arterial glucose was 0.9 ± 0.9 milligrams per 100 ml. These values for venous glucose were 0.8 ± 0.8 milligrams per 100 ml., and the values for A-V difference were 1.2 ± 1.25 milligrams per 100 ml.

DISCUSSION

The data show that the femoral A-V glucose difference in the resting fasting state is usually small. Statistically, 95 per cent of values should fall within two standard deviations around the arithmetic mean. In a study of A-V differences, two different values are determined in order to calculate the difference. It is, therefore, theoretically possible that the A-V difference be equivalent to four standard deviations and still not be outside the limits of error of the method. In

this study, the standard deviation of the method was ± 0.47 milligrams per 100 ml., and one has confidence in A-V differences greater than two milligrams per 100 ml. If it is postulated that in some instances there is actually no uptake of glucose by the tissues of the leg, the negative differences recorded in Table II are of no significance, as they fall well within the theoretical limit of two milligrams per 100 ml. It is concluded that while a small extraction of glucose usually occurs, frequently no demonstrable amount of sugar is being removed.

Among others, Somogyi,[7] Bose,[8] and Rabinowitch[9] report A-V glucose differences across the arm in normal subjects. They fail to show any negative values and tend to have a higher average difference than here reported. Glassberg[10] in 1930 said there was general agreement that the resting A-V difference is three to five milligrams per 100 ml. Others have considered the difference as being negligible although their data show considerable range.

The amount of glucose used by the lower extremities in the resting fasting state has never been estimated. Indirect observations indicate that it is low. Myers[2] observed that in man of average size (1.73 square meters), the liver discharged into the hepatic vein approximately 112 milligrams of glucose per minute. The brain, from the studies of Scheinberg and Stead,[1] uses approximately 78 milligrams per minute. Thus, there are roughly only 34 milligrams of glucose per minute available for the rest of the body. Goodale and associates[3] showed that in man the heart used negligible amounts of glucose when the general blood level is below 80 milligrams per cent.

If one assumes that the lower extremities compose 40 per cent of the body volume, one can make a rough calculation of the glucose consumption. From other studies[4] of peripheral blood flow as measured in the plethysmograph at 32° C. and room temperature of 25° C., the average blood flow in the calf was 1.4 ml. per 100 ml. of tissue per minute. As the room temperature usually exceeds 25° C., it seems reasonable to double this figure to allow a calculation of the upper limit for the average fasting glucose removal by the lower extremities. In a 70-kilogram man, the lower extremity volume is approximately 28 liters, and the total blood flow based on a rate of blood flow of 2.8 ml. per 100 ml. per minute is 780 ml. per minute. With an average A-V glucose difference of 2.2 milligrams per 100 ml., the fasting glucose uptake by the lower extremities would be 17 milligrams per minute. This rate amounts to approximately 24.5 grams per twenty-four hours.

SUMMARY

The titration method of Somogyi for glucose determination has been evaluated and shown to yield a high degree of sensitivity and reproducible accuracy at blood sugar levels below 120 milligrams per 100 ml.

The femoral arterial and venous glucose concentrations and the A-V differences have been shown to remain remarkably constant in the resting, fasting state during periods of two through twenty-five minutes.

The average femoral A-V glucose difference is 2.2 milligrams per 100 ml. Frequently there is no measurable difference.

There is no correlation between A-V glucose and A-V oxygen differences across the leg.

A rough estimation of the total uptake of glucose by the resting lower extremities during the fasting state has been calculated not to exceed 17 milligrams per minute.

REFERENCES

1. Scheinberg, P., and Stead, E. A., Jr.: Cerebral Blood Flow in Male Subjects as Measured by the Nitrous Oxide Technique. Normal Values for Blood Flow, Oxygen Utilization, Glucose Utilization, and Peripheral Resistance with Observations of the Effect on Tilting and Anxiety, J. Clin. Investigation **28:** 1163, 1951.
2. Myers, J. D.: Net Splanchnic Glucose Production in Normal Man and in Various Disease States, J. Clin. Investigation **29:** 1421, 1950.
3. Goodale, W. T., Olson, R. E., and Hackel, D. B.: The Effect of Fasting and Cardiac Failure upon Heart Muscle Metabolism in Man, J. Clin. Investigation **29:** 816, 1950.
4. Abramson, D. I., and Fierst, S. M.: Resting Blood Flow and Peripheral Vascular Responses in Hypertensive Subjects, Am. Heart J. **23:** 84, 1942.
5. Somogyi, M.: A New Reagent for the Determination of Sugars, J. Biol. Chem. **160:** 61, 1945.
6. Somogyi, M.: Determination of Blood Sugar, J. Biol. Chem. **160:** 69, 1945.
7. Somogyi, M.: Studies of Arteriovenous Differences in Blood Sugar. I. Effect of Alimentary Hyperglycemia on the Rate of Extra-hepatic Glucose Assimilation, J. Biol. Chem. **174:** 189, 1948.
8. Bose, J. P.: Arteriovenous Sugar Differences in Diabetes Mellitus: Its Significance in Adjudging the Severity of the Disease, Indian J. Med. Res. **23:** 1, 1935-36.
9. Rabinowitch, I. M.: Simultaneous Determinations of Arterial and Venous Blood Sugars in Diabetic Individuals, Brit. J. Exper. Path. **8:** 76, 1927.
10. Glassberg, B. Y.: The Arteriovenous Difference in Blood Sugar Content, Arch. Int. Med. **46:** 603, 1930.
11. Hickam, J. B., and Frayser, R.: Spectrophotometric Determination of Blood Oxygen, J. Biol. Chem. **180:** 457, 1949.

RADIATION REACTIONS IN PARABIOTIC RATS

JOSHUA L. EDWARDS, M.D., AND SHELDON C. SOMMERS, M.D.
BOSTON, MASS.

IN AN effort to determine whether tissue effects of x-radiation might be mediated by circulating humoral substances, a series of parabiotic rats was prepared. After irradiation of one partner, leukocyte counts and histologic examination of tissues of pairs sacrificed at intervals provided material for evaluating radiation reactions in each partner. Because of the importance of adrenal function in homeostasis, adrenalectomy of either partner was done before irradiation in some pairs.

MATERIALS AND METHODS

Preparation of Parabionts.—Two hundred eighty-four albino rats of Slonaker strain, 30 to 60 days old, were paired by littermates and as to sex and weight. Parabiosis was achieved by modified Bunster-Meyer operative technique[1, 2] under intraperitoneal phenobarbital anesthesia.

An incision was made from near the base of the ear to near the base of the tail on adjoining sides of two animals. Skin flaps were slightly undermined, and the ventral flaps sewed together with 0000 chromic catgut. The peritoneal cavities were opened by linear incisions, and the corresponding edges approximated with 0000 plain catgut, forming a celioanastomosis. A single mattress suture joined the two scapulae, and the dorsal skin flaps were then sewed together, completing the anastomosis.

Hemiadrenalectomy.—Four hundred comparable rats were joined by the same operative procedure. At the same time one adrenal of one animal was removed through a short flank incision. Four to seven days later the remaining adrenal of the same animal was similarly removed. One partner was thus completely adrenalectomized.

The parabiosis operation may be followed by various complications. Operative shock, poor recovery from anesthesia, pneumonia, infection of the operative wounds, or failure of healing may occur. Older rats are poor material for parabiosis, partly because they may fight. If littermates were not employed, various indications of tissue incompatibility became prominent. Animals which died spontaneously post irradiation without prompt autopsy were discarded (Table I).

Irradiation.—Twelve to twenty-five days following the parabiotic operation the right member of each parabiotic pair received a single exposure of 400, 800, 1,200 or 1,600 r. of total body radiation. The left partner was carefully protected by a lead shield (Fig. 1), accurately aligned, and tested by placing film badges under the shield. An insignificant amount of contaminating scatter radiation was found. Single control rats were irradiated with the same doses.

A GE Maximar x-ray machine was employed, with the following radiation factors: 200 kv., 10 Ma., 35 cm. target distance, no filter, half-value layer equivalent to 0.35 mm. of copper, administered at 155 r. per minute.

After irradiation parabionts were sacrificed at intervals of one hour to twenty-nine days with 400 r. x-radiation, one hour to twenty-one days after 800 r., twelve hours to eight days after 1,200 r., and one hour to four days after 1,600 r.

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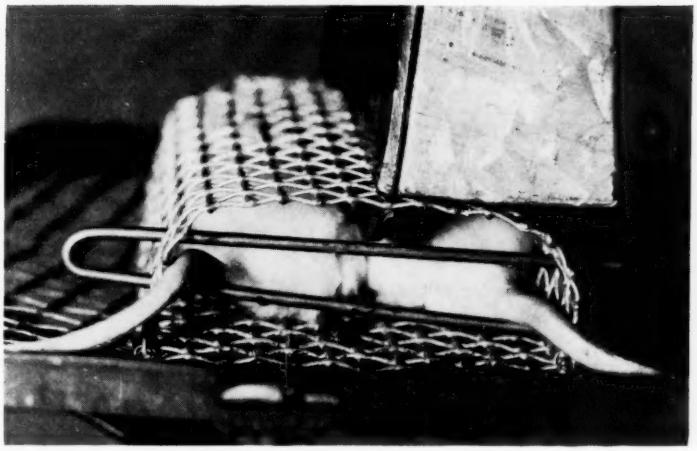

Fig. 1.—Parabiotic rats in position for irradiation of the right partner, with protection of left partner by the lead shield and the metal divider shown.

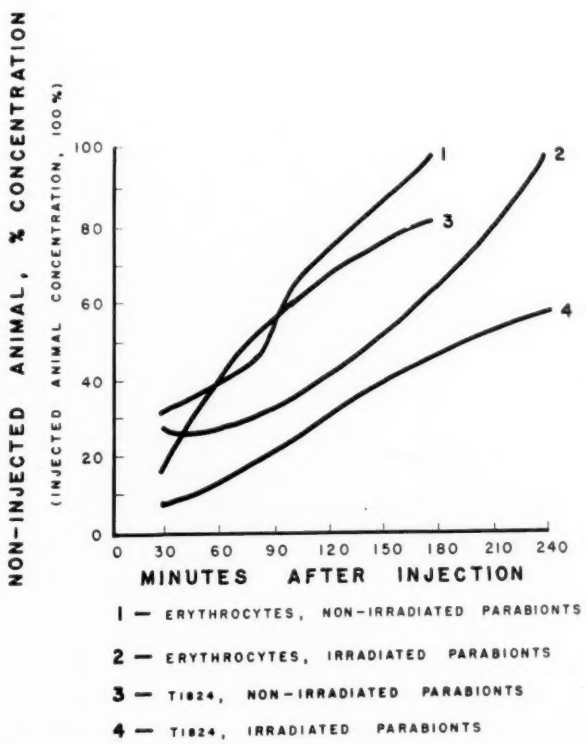

Fig. 2.—Rates of transfer of P^{32} labeled erythrocytes and T-1824 dye between parabiotic rats, nonirradiated and postirradiation.

TABLE I. DISPOSITION OF ALL ANIMALS

	NORMAL PARABIONTS (PAIRS)	ADXED PARABIONTS (PAIRS)
Irradiated and autopsied	48	74
Died after irradiation, not autopsied	5	3
Controls, not irradiated	9	5
One member died from the operative procedure	35	22
One member died before irradiation	35	89
Died because of fighting	8	
Evisceration of wound	1	5
Used for preliminary dye studies	2	
Practice cases	3	
Shielded member contaminated by radiation		1
Adrenal not completely removed		1
Total*	146	200

*Does not include eight parabionts used in exchange experiment or a small number irradiated with 1200 r.

Vascular Exchange.—Demonstration of adequate cross circulations between the parabiotic partners was achieved by measuring the rates of transfer of erythrocytes labeled with P^{32}, and plasma albumin combined with Evans Blue dye (T-1824). Measurements were made on unirradiated parabionts and pairs exposed to 1,600 r., four to twenty-four hours previously.

Normal rat blood was separated into cells and plasma. Erythrocytes were incubated with a solution containing P^{32} for forty-five minutes, washed and resuspended in physiologic saline. A measured amount of Evans Blue dye (T-1824) was added, and the combined suspension injected intravenously into the right members of four normal and four irradiated

TABLE II. EXCHANGE OF BLOOD AND ERYTHROCYTES IN PARABIONTS

NONIRRADIATED PARABIONTS

ANIMAL	INTERVAL FOLLOWING INJECTION (MIN.)	CONC. OF T-1824*		CONC. OF ERYTHROCYTES	
		ABSOLUTE VALUE	RELATIVE PER CENT† L OF R	COUNTS PER MINUTE	RELATIVE PER CENT‡ L OF R
R1	30	0.36	—	675	—
L1	30	0.06	16	229	33
R2	90	0.18	—	1,251	—
L2	90	0.09	50	513	41
R3	125	0.32	—	1,797	—
L3	125	0.21	66	1,288	72
R4	180	0.18	—	2,523	—
L4	180	0.14	78	2,757	100

IRRADIATED PARABIONTS (1,600 R. TO THE RIGHT MEMBER; RIGHT MEMBER INJECTED)

ANIMAL	INTERVAL FOLLOWING RADIATION (HR.)	INTERVAL FOLLOWING INJECTION (MIN.)	CONC. OF T-1824		CONC. OF ERYTHROCYTES	
			ABSOLUTE VALUE	RELATIVE PERCENTAGE L OF R†	COUNTS PER MINUTE	RELATIVE PERCENTAGE‡ L OF R
R5	6	30	0.96	—	2,057	—
L5	6	30	0.08	8.3	606	29
R6	6	60	0.32	—	1,050	—
L6	6	60	0.04	12.0	304	29
R7	4	120	0.22	—	251	—
L7	4	120	0.06	30.0	99	40
R8	4	240	0.125	—	525	—
L8	4	240	0.071	56.0	528	100

*Concentration of T-1824 expressed in relative units and without quantitative significance

†Relative percentage is the percentage of concentration of albumin-bound dye and P^{32} labeled erythrocytes in blood of noninjected animal compared to blood of injected animal.

‡Controls: Supernatant plasma of all samples checked for radioactive material. None found.

parabiotic pairs. At intervals from thirty minutes to four hours blood was withdrawn from each partner, and the relative concentrations of P^{32} and Evans Blue dye determined in the two members (Table II and Fig. 2). The results were of the same order of magnitude as found by Huff, Trautman, and Van Dyke,[3] who reported an average 0.66 per cent of blood volume transferred per minute across the anastomosis in rats.

Leukocyte Counts.—Both before and after irradiation white cell counts of both partners were made, particularly as a check of proper radiation exposures. Counts also served as a semiquantitative index of responses to radiation. Individual irradiated rats were used as controls showing the leukopenic response. Rats with complicating infections or inflammation had leukocytosis and were excluded.

Histologic Studies.—Complete autopsies were performed on all animals sacrificed, and numerous microscopic sections prepared. Tissues routinely obtained for microscopic study included heart, lung, spleen, pancreas, tongue, forestomach, intestine, liver, adrenal, kidney, bladder, testis or ovary, lymph node, thyroid, parathyroid, salivary gland, thymus, brain, eye, and bone marrow. In some cases the oviduct and uterus were examined. Trachea and esophagus were demonstrated in the thyroid block. Tissues were usually fixed in Zenker-formol and stained with hematoxylin and eosin. Bone marrow was stained by the Giemsa method. A few tissues were frozen dried and vacuum embedded in paraffin.

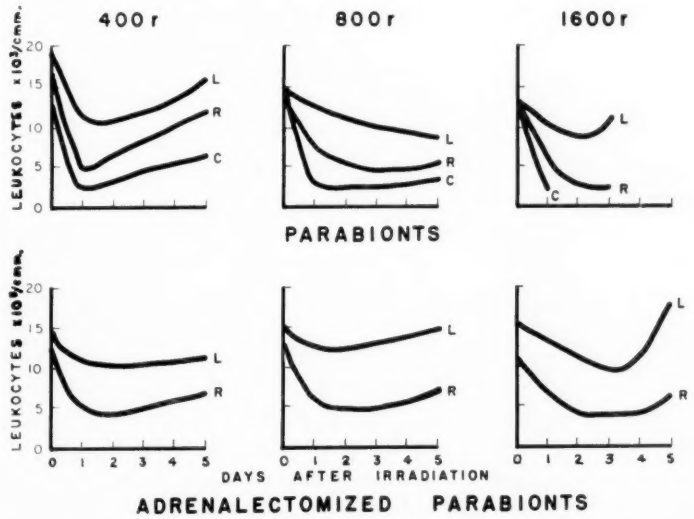

Fig. 3.—Mean postirradiation leukocyte counts of protected left parabiont (*L*), irradiated right parabiont (*R*), and single irradiated control rats (*C*). Animals with sepsis excluded.

All tissues of irradiated and protected parabiotic partners and controls were systematically investigated for pathologic alterations, particularly those of radiation reaction. To avoid bias, correlations of dosages and postirradiation time intervals were not made until the histologic study was completed.

RESULTS

Leukocyte Response.—In terms of the leukopenic responses to radiation, the parabionts showed an increased resistance compared to single rats. The white cell counts of the irradiated partners did not fall as low as single rats exposed to the same dose, and leukocyte levels rose sooner post irradiation. The milder leukopenia and the subsequent leukocytosis of parabionts indicated that leukocytes were crossing the anastomosis and supporting the white-cell counts of the irradiated partners. Fig. 3 shows the comparative responses of mean

leukocyte counts of the parabiotic partners and controls. After large doses of radiation, peripheral vessels were terminally so contracted and blood clotted so quickly as to prevent collecting significant numbers of control counts.

Histologic Responses of Irradiated Animals.—In irradiated parabionts and single irradiated controls closely comparable microscopic acute effects of irradiation were observed, which did not differ from experimental radiation reaction, as previously described by many workers.[4-10] Nuclear pyknosis and swelling, cell fragmentation, cessation of proliferative activity, and disarrangement of normal architecture were particularly striking in lymph nodes, thymus, splenic corpuscles, and lymphoid aggregates in intestinal crypts, bone marrow, and hematopoietic foci normally found in the rat spleen; and in developing ovarian follicles or testicular spermatogenic cells.

Less striking but definite damage was observed in tongue, forestomach, stomach, bladder, salivary gland, and eye. The covering epithelium of ventral tongue surface, forestomach, and bladder showed nuclear swelling, distortion, or vacuolization of basal and partly matured cells, with focal pyknosis. Stomach mucosa, particularly after 1,200 or 1,600 r., showed variable decolorization, granularity, or vacuolization of parietal cells, with some distortion of their shape.[11] Eosinophilic ducts of salivary glands were altered by irregular pyknotic destruction of individual cells, at times with irregular dilatation of lumens. Similar focal changes were observed in pancreatic ducts. The eye, in the lens capsule epithelium near the anterior bow, had focal pyknotic fragmented nuclei,[12] and the inner retinal layers often were edematous, with rare pyknotic nuclei.

Endothelial cells of arteries, arterioles, and capillaries showed acute change, most frequently observed in lungs, pancreas, kidneys, and connective tissues. It consisted in prominent endothelial vacuolization, which at times also involved cells deeper in the vessel walls. Endothelial nuclei often became pyknotic, and lining cells appeared to slough. Glomerular capillaries participated in these changes. As applied to larger vessels, these changes have been termed acute alterative arteritis.[13] Because of the limited morphologic alterations of which vascular components are capable, they are seen after many different noxious stimuli.

Aside from this vascular reaction, the heart, liver, adrenal, kidney, thyroid, parathyroid, and brain demonstrated no histologic changes ascribable to radiation. Pancreatic acinar cells and salivary gland cells of serous type had increased apical cytoplasmic eosinophilia associated with loss of zymogen granules. This has been found by others in the "alarm reaction."[14] Mucus-producing cells of salivary gland and large intestine showed increased activity, considered a nonspecific catarrhal response. Only in exceptional cases a few thyroid-cell nuclei were noted to be pyknotic or irregularly swollen.

Post irradiation there was frequently a striking interstitial edema of tracheal, bronchial, and peribronchial tissues, followed later by inflammation. Overlying epithelium showed varying degrees of degeneration, which was also present in the tongue. This appeared quantitatively sufficient to produce

TABLE III. TISSUE HYPERPLASIAS IN PROTECTED PARTNERS, ONE TO FIVE DAYS POST IRRADIATION

	NONIRRADIATED PARABIOTIC RAT PARTNERS		
HYPERPLASIA OF:	NORMAL	PARTNER ADRENALECTOMIZED	ADRENALECTOMIZED
Intestinal epithelium	2/14 (14%)	13/26 (50%)	16/33 (49%)
Bone marrow	9/15 (60%)	19/26 (73%)	29/32 (91%)
Splenic hematopoiesis	10/17 (59%)	14/24 (58%)	15/31 (48%)
Lymph node	7/14 (50%)	21/26 (81%)	29/34 (85%)
Thymus	4/12 (33%)	9/25 (36%)	26/31 (84%)
Ovarian granulosa cells	6/8 (75%)	10/12 (83%)	13/17 (77%)
Testicular spermatocytes	6/9 (67%)	8/13 (62%)	11/17 (65%)

respiratory distress. It seems to explain the morbidity and mortality of experimental irradiation confined to the head, neck, and thorax.[15] Recently stenosing end results have been reported in mice.[16]

Incidental pathologic conditions found in some rats included parasites in intestine, bladder, and occasionally liver; and evidence of bacterial and virus infections, as shown by focal inflammation of lung, liver, or kidney frequently associated with inclusion bodies. Following high doses of irradiation, ova of the intestinal nematodes showed degenerative changes.

Histologic Responses With Respect to Dose.—The differences in radiation reactions associated with doses of 400, 800, 1,200, or 1,600 r. were quantitatively very similar to those described by other workers. With higher doses the sensitive tissues were more uniformly and thoroughly damaged, and changes were more frequently observed in more resistant organs like tongue, forestomach, stomach, bladder, salivary gland, and eye. With 400 or 800 r., after three to four days, regenerative cellular foci were present in lymph nodes, intestinal crypts, bone marrow, and gonads. These were scarcer after 1,200 or 1,600 r., and their appearance was delayed.

Radiation reaction per se did not appear altered by parabiosis. Only the increased postirradiation longevity of some pairs allowed tissues a longer interval for radiation degeneration and regeneration than occurred in irradiated single animals.[17]

Histologic Responses in Nonirradiated Partners.—The nonirradiated parabionts showed no microscopic evidence of radiation reaction mediated by circulating substances. Since the pairs had a common peritoneal cavity, and intestinal loops were easily herniated across the juncture, occasionally observed radiation reaction limited to the small intestine of the protected partners was not unexpected.

Histologic variations from normal observed in some protected partners included one- to five-day periods of hyperplasia of those tissues most damaged

TABLE IV. TESTICULAR INTERSTITIAL CELL HYPERPLASIA, 1 TO 5 DAYS POST IRRADIATION

	IRRADIATED RAT	PROTECTED RAT
Normal parabionts	0/9	0/9
Irradiated rat adrenalectomized	8/13 (61%)	6/13 (46%)
Nonirradiated rat adrenalectomized	1/17 (6%)	14/17 (83%)
Totals: Adrenalectomized	22/30 (73%)	
Partner adrenalectomized	7/30 (23%)	

in the irradiated partners (Table III). In the small intestine mitoses were present in large numbers in crypt epithelium, seemingly increased for three or four days post irradiation. The crypt nuclei were prominent, appeared enlarged, crowded, and sometimes slightly irregularly palisaded (Fig. 4). For the same interval bone marrow and splenic hematopoietic hyperplasia developed, with increased numbers of less mature and stem cells (Fig. 5). In lymphoid tissues germinal centers were enlarged, with enhanced lymphopoiesis, and in thymus the cortex was thickened (Fig. 6). These hyperplasias were more regularly observed in hemiadrenalectomized parabionts (Table III).

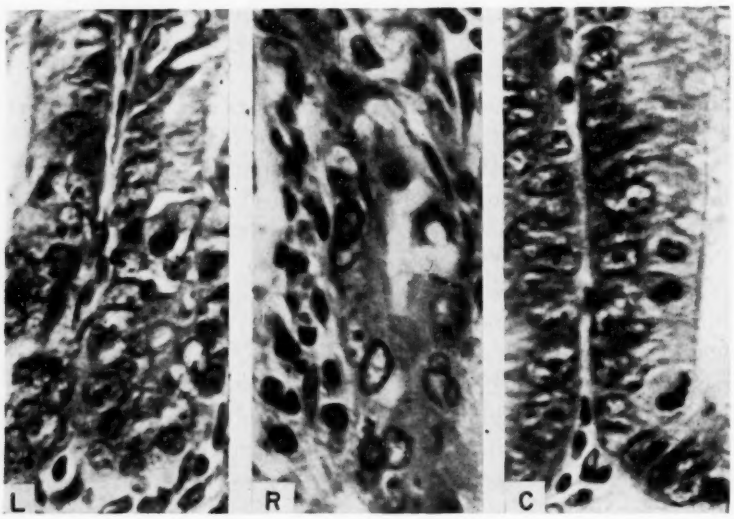

Fig. 4.—Small intestinal epithelium of unirradiated left partner (*L*) on the left, irradiated right parabiont (*R*) in center, and normal control rat (*C*) on the right. Enlargement and crowding of crypt nuclei of *L* and radiation damage and atrophy of *R* are present two days after 1600 r. (×825.) Hematoxylin and eosin.

Granulosa cells of developing ovarian follicles were frequently layered in increased rows and showed numerous mitoses. The central follicular cavities were reduced in size (Fig. 7). Spermatogenic cells in testicular tubules, particularly primary and secondary spermatocytes, were enlarged, mainly by increase in cytoplasmic volume, so that they filled more of the tubular lumens. Maturation to spermatids appeared decreased, and there were increased amounts of cytoplasmic material free in the spermatic tubules (Fig. 8). In both sexes these alterations did not subside as quickly as the other tissue hyperplasias, and were evidently not affected by hemiadrenalectomy (Table III). This analysis is complicated by other endocrine interactions which also occur in nonirradiated parabionts.

Histologic Responses With Respect to Adrenalectomy.—Histologic effects of radiation in hemiadrenalectomized parabiotic rats were quantitatively and qualitatively no different from other parabionts. This was true whether the irradiated or protected partner had been adrenalectomized. Perhaps the easiest

way to distinguish the adrenalectomized partners, whether irradiated or protected, was by the increased eosinophilic leukocytes in tissues, particularly in intestinal lamina propria, bone marrow, and sometimes spleen.

Lymphoid tissue was hyperplastic in most of the nonirradiated members of hemiadrenalectomized pairs (83 per cent), regardless of which rat was adrenalectomized. The frequency and degree of lymphoid hyperplasia were significantly greater than in normal parabionts (Table III).

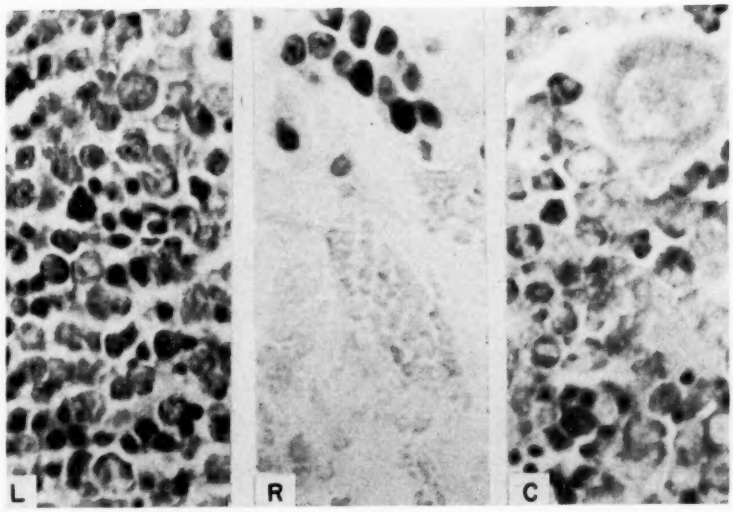

Fig. 5.—Bone marrow sections of protected (*L*), irradiated (*R*) parabionts, and normal control rat (*C*), reading left to right. Hyperplasia of protected parabiont's hematopoietic cells (*L*), radiation atrophy of its partner's marrow (*R*), five days after 1600 r., and normal pattern (*C*) are shown. (×825.) Hematoxylin and eosin.

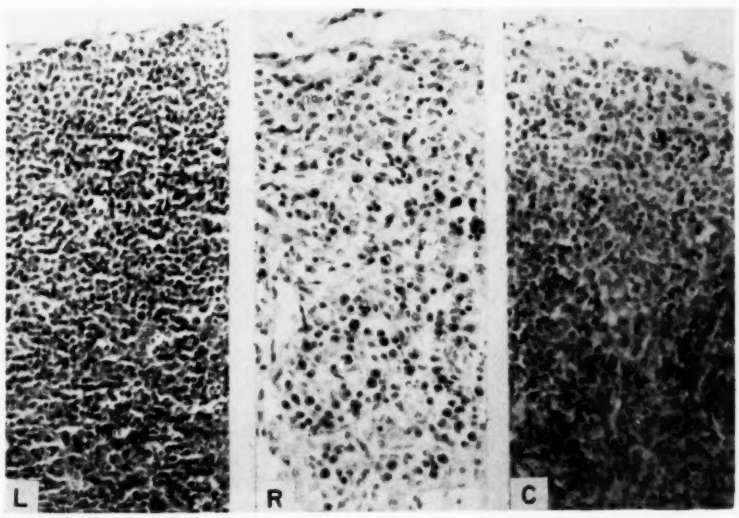

Fig. 6.—Submental lymph nodes of protected (*L*) and irradiated parabionts (*R*), two days after 1600 r. The former is hyperplastic and the latter shows radiation atrophy, compared with the normal control rat (*C*). (×205.) Hematoxylin and eosin.

Testicular interstitial cells were often hyperplastic (73 per cent) in the adrenalectomized partners, and less regularly (23 per cent) in the opposite members of the pairs (Table IV). Rarely, ovarian hilar cells were similarly increased.

In hemiadrenalectomized parabionts there was an inconstant increase in recognizable tissue mast cells, particularly observed in striated muscle of tongue

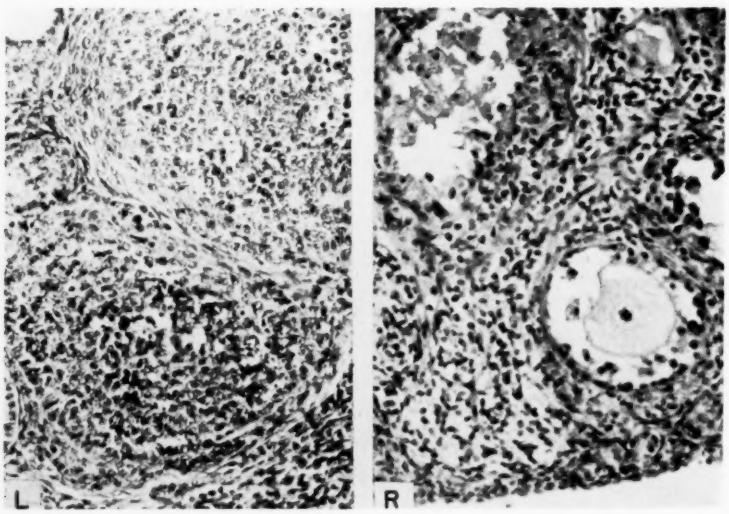

Fig. 7.—Protected parabiont ovary (*L*) with granulosa-cell hyperplasia, compared to its irradiated rat partner (*R*), three days after 1,200 r, showing marked radiation damage to ova and granulosa cells. (×205.) Hematoxylin and eosin.

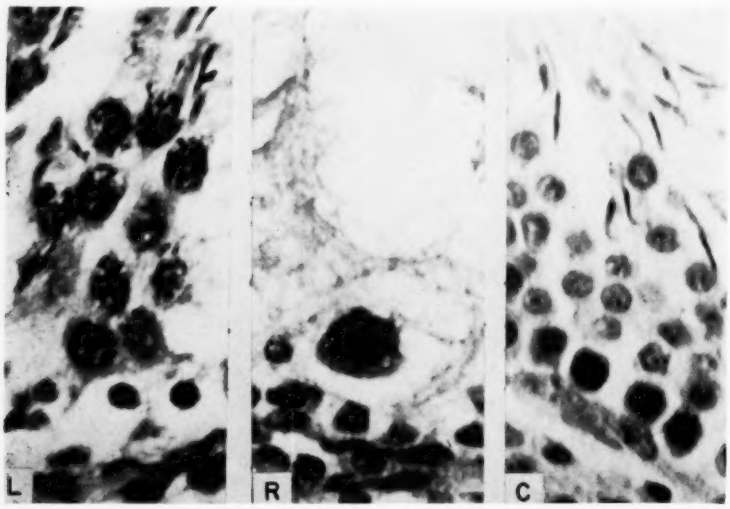

Fig. 8.—Unirradiated parabiont testis (*L*) with enlargement and hyperplasia of immature spermatogenic cells, associated with decreased spermatid maturation. The irradiated partner (*R*), one and one-half days after 1,600 r, has typical radiation damage of testis. Control rat (*C*) shows normal spermatogenesis. (×825.) Hematoxylin and eosin.

and in bone marrow, from one to four days post irradiation. It occurred in either partner, without uniform relationship to which one had been adrenalectomized or irradiated.

All hemiadrenalectomy experiments in parabiotic rats were complicated by the rat's ability to form accessory adrenal cortical tissue, or "glandular fat."[18, 19] Abundant masses appeared in the renal pericapsular and peripelvic fat, and at the ovarian hilus. It was increased in the mediastinum at the base of the heart, around the thymus, and in the cervical region outside the capsules of thyroid and salivary glands.

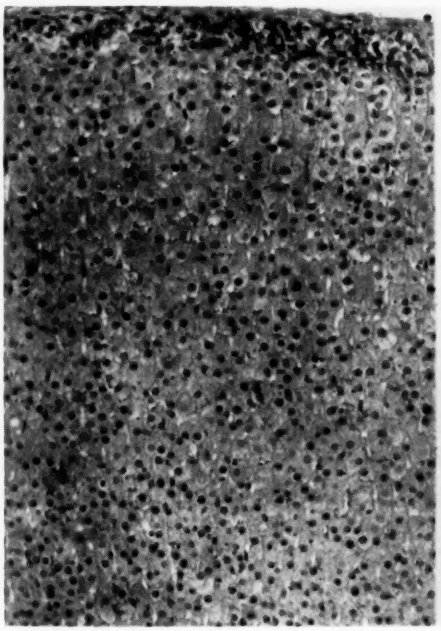

Fig. 9.

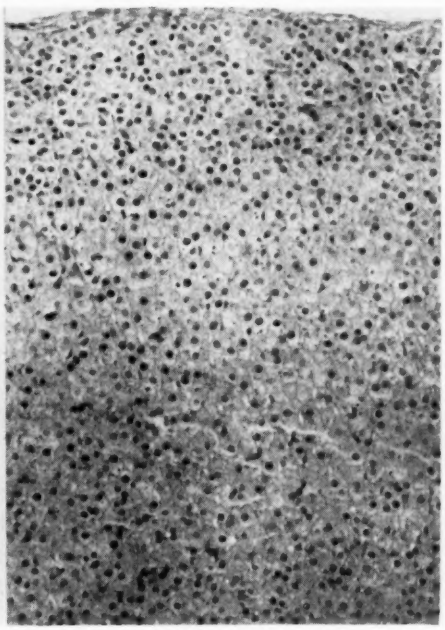

Fig. 10.

Fig. 9.—Adrenal from irradiated rat parabiont, two days after 1,600 r. Its partner had been adrenalectomized. Cortical cells of zona fasciculata are enlarged and depleted of lipoid. (×165.) Hematoxylin and eosin.

Fig. 10.—Adrenal of protected rat parabiont whose partner was both adrenalectomized and irradiated with 800 r, one and one-half days before. Cortical cells are hypertrophied and contain abundant fine cytoplasmic vacuoles. (×165.) Hematoxylin and eosin.

When the nonadrenalectomized partner was irradiated, its adrenals showed microscopic changes not otherwise apparent. The hyperplastic zona fasciculata cells lost a moderate amount of finely vacuolated lipoid from their hypertrophied cytoplasms, which was replaced after four to five days (Fig. 9). This change has been reported in single rats as a reaction to acute stress.[20] It was not observed when the adrenals were left in the protected partner (Fig. 10). In protected partners thymic and testicular interstitial-cell hyperplasias were more common (84 and 82 per cent) when the rat with adrenals was irradiated than when the adrenalectomized partner was irradiated (36 and 46 per cent). This indicates indirectly that radiation depressed adrenal cortical function (Tables III and IV).

DISCUSSION

Microscopic observations made in this study provided no evidence for the transmission of radiation effects by circulating substances. If there were such substances their existence in rats would appear to have been so fugitive or their influences so subtle that recognizable morphologic alterations did not result at the time intervals studied.[17]

The tissue destruction following radiation damage at times had recognizable repercussions in the protected parabiotic partners. Cells which normally circulate, particularly leukocytes, demonstrated this interaction. In the irradiated members normal resupply of leukocytes was prevented after bone marrow and lymphoid damage, but the falling white blood cell counts were bolstered by the protected partners' leukocytes. Increased over-all demand was indicated by the hematopoietic and lymphoid hyperplasias, of which the protected partners were immediately capable. Participation of substances such as leukotaxin and leukocyte promoting factor is inferred.[21] Nuclear breakdown products are also known to enhance hematopoiesis.[22] Because of convenient quantitative techniques, such hematologic reactions have had wide study.

Small intestinal epithelium showed a similar post-irradiation hyperplasia in some protected rat partners. Destruction of intestinal cells apparently set off a reparative stimulus to which the normal intestine was capable of responding more quickly than irradiated bowel. Evidently this was mediated by circulating factors, whether trephones, hormones, or of other types.

Hyperplasia of ovarian granulosa cells would seem to be a third instance of the same type of reaction. Here other experimental work indicates that increased pituitary follicle-stimulating hormone secretion probably has resulted from loss of inhibiting estrogen when irradiation damaged the granulosa and theca cells.[6, 23, 24] The protected members' granulosa cells were capable of immediate proliferative response.

In the testis a somewhat comparable hypertrophy and hyperplasia at the spermatocyte level occurred in nonirradiated parabiont partners. At the expense of further differentiation these immature cells enlarged and cast off cytoplasm into the spermatic tubules. Since interstitial cells are quite radioresistant, and major testicular irradiation damage was in tubules, this indicated indirect effects produced by circulating substances of spermatogenic cell origin. It is another demonstration that some testicular tubular cells have hormonal activity.[25, 26]

Hemiadrenalectomy did not affect the localization, severity, or microscopic appearance of radiation reactions. The outstanding morphologic evidences of adrenal removal included lymphoid hyperplasia, tissue eosinophilia, hyperplasia of testicular interstitial cells, and compensatory production of glandular fat. When adrenals remained in the irradiated partner, loss of adrenal cortical lipoid not otherwise observed became evident. By increased incidences of lymphoid and interstitial cell hyperplasias it was independently indicated that irradiation had reduced adrenal cortical function. The various tissue changes

just listed are acknowledged morphologic evidences of adrenal cortical exhaustion or insufficiency. No changes ascribable to adrenal medulla removal were seen.

It is of interest that hyperplasias of protected intestinal epithelium and bone marrow were more frequent in hemiadrenalectomized parabionts. The simplest satisfactory explanation of this would seem to be that two functioning anterior pituitary glands tended to outweigh in their repair-stimulating hormonal activities post irradiation the inhibitory effects upon protein anabolism of one set of adrenal glands. That this is not also true of gonadal tissues shows that separate hormone systems are involved, as is already well known.

The "alarm reaction," with its implication of pituitary-adrenal-cortical responsibility for major adaptive reactions to noxious agents, might be invoked to explain many of the changes described. Lymphoid tissue destruction, depletion of zymogen granules in salivary gland and pancreas, and decreased adrenal cortical lipoid are characteristic findings in reactions to stress. However, the acknowledged imperceptible insidious nature of ionizing radiation makes it unlike ordinary stress-producing agents. Ionizing radiation does not fit easily into a list of causes of "alarm reactions." Radiation has in common with these miscellaneous noxi mainly a striking transient interference with protein resynthesis, particularly of nucleoproteins, following tissue destruction. Inhibition of protein and nucleoprotein synthesis may be effected directly by ionizing radiation, or indirectly through pituitary-adrenal stimulation, or otherwise.

The complexity of experimental parabiont reactions should not be overlooked. In such animals blood and tissue incompatibilities were always potential and sometimes obvious complicating factors. Other types of endocrine imbalance were also likely. For purposes of unity these matters are considered separately, since they had no crucial influence on the radiation changes, and also were mostly only incidentally altered by irradiation.

SUMMARY

A study was made of morphologic tissue changes and leukocyte counts after ionizing radiation, using parabiotic rats. One member was protected from x-rays by a lead shield, and the other member given a single dose of 400, 800, 1,200, or 1,600 r. total body x-radiation. Vascular connections were proved using P^{32} labeled erythrocytes and T-1824 dye.

No pathologic evidence of radiation damage to the protected rats was found, such as might be mediated by circulating substances. Increased resistance of parabionts to irradiation of one member was indicated by less marked leukopenia than in irradiated single rats, and the protected rats' leukocytes appeared to bolster the irradiated partners.

Intestinal, lymphoid, and hematopoietic tissues of the protected partners often showed brief periods of hyperplasia post irradiation. This was more frequently observed in hemiadrenalectomized parabionts. Ovarian granulosa cells and testicular spermatocytes also sometimes became hyperplastic in protected rats without relation to adrenalectomy. No differences in radiation reactions

were observed ascribable to adrenalectomy. Ionizing radiation damage appears to have in common with the "alarm reaction" mainly an inhibition of protein resynthesis following increased tissue breakdown.

Acknowledgment is made to the staff of the Cancer Research Institute, New England Deaconess Hospital for advice and technical assistance with irradiation, biology, hematology, and histopathology. Misses Rosanna Chute and Anne E. Grimwade performed many operations and made the blood counts.

REFERENCES

1. Bunster, E., and Meyer, R. K.: Improved Method of Parabiosis, Anat. Rec. **57**: 339-343, 1933.
2. MacMillan, J. C.: Unpublished experiments.
3. Huff, R. L., Trautman, R., and Van Dyke, D. C.: Nature of Exchange in Parabiotic Rats, Am. J. Physiol. **161**: 56-74, 1950.
4. Warren, S. L., and Whipple, G. H.: Roentgen Ray Intoxication. I. Unit Dose Over Thorax Negative—Over Abdomen Lethal. Epithelium of Small Intestine Sensitive to X-Rays, J. Exper. Med. **35**: 187-202, 1922.
5. Henshaw, P. S.: Experimental Roentgen Injury. I. Effects on the Tissues and Blood of C3H Mice Produced With Single Small Whole-Body Exposures. Experimental Roentgen Injury. II. Changes Produced With Intermediate-Range Doses and a Comparison of the Relative Susceptibility of Different Kinds of Animals, J. Nat. Cancer Inst. **4**: 477-484, 485-501, 1944.
6. Bloom, W., ed.: Histopathology of Irradiation From External and Internal Sources, New York, 1948, McGraw-Hill Book Co., Inc., 808 pp.
7. Tullis, J. L.: The Response of Tissue to Total Body Irradiation, Am. J. Path. **25**: 829-851, 1949.
8. Warren, S., MacMillan, J. C., and Dixon, F. J.: Effects of Interaal Radiation of Mice With P^{32}. I. Spleen, Lymph Nodes, Thymus, Bone and Bone Marrow: II. Gonads, Kidneys, Adrenal Glands, Digestive Tract, Spinal Cord, Lungs, and Liver, Radiology **55**: 375-389, 557-570, 1950.
9. Warren, S., and Bowers, J. Z.: The Acute Radiation Syndrome in Man, Ann. Int. Med. **32**: 207-216, 1950.
10. Lawson, B. G., and Tullis, J. L.: The Progression of Morphologic Lesions in Swiss Mice Exposed to 625 r., 2,000 KVP, Total Body X-radiation, Military Surgeon **109**: 281-293, 1952.
11. Palmer, W. L., and Templeton, F. E.: Effect of Radiation Therapy on Gastric Secretion, J.A.M.A. **112**: 1429-1434, 1939.
12. Wilder, H. C., and Maynard, R. M.: Ocular Changes Produced By Total Body Irradiation, Am. J. Path. **27**: 1-19, 1951.
13. Karsner, H. T.: Acute Inflammations of Arteries, Springfield, Ill., 1947, Charles C Thomas, 37 pp.
14. Ehrich, W. E., and Seifter, J.: Role Played By the Salivary Glands in the "Alarm Reaction," Arch. Path. **45**: 239-245, 1948.
15. Bender, A. E.: Experimental X Irradiation of the Rat Thyroid, Brit. J. Radiol. 21: 244-248, 1948.
16. Silberberg, R., Silberberg, M., and Dixon, F. J.: Obliterating Tracheitis, A Complication Following Administration of Radioactive Iodine, J. LAB. & CLIN. MED. **39**: 256-259, 1952.
17. Barnes, W. A., and Furth, D. B.: Studies on the Indirect Effect of Roentgen Rays in Single and Parabiotic Mice, Am. J. Roentgenol. **49**: 662-681, 1943.
18. Mayeda, T.: Untersuchungen über Parabiose mit besonderer Berücksichtigung der Transplantion und Hypernephrektomie, Deutsche Ztschr. f. Chir. **167**: 295-348, 1921.
19. Laqueur, G. L., and Harrison, M. B.: Glandular Adipose Tissue Associated With Cytotoxic Suprarenal Contraction and Diabetes Mellitus, Am. J. Path. **27**: 231-245, 1951.
20. Selye, H., and Stone, H.: On the Experimental Morphology of the Adrenal Cortex, Springfield, Ill., 1950, Charles C Thomas, 105 pp., 49-63.
21. Menkin, V.: New Concepts of Inflammation, Springfield, Ill., 1950, Charles C Thomas.
22. Doan, C. A.: Nucleinate-Induced Extramedullary Myelopoiesis, Proc. Soc. Exper. Biol. & Med. **29**: 1030-1035, 1931-32.
23. Furth, J., and Boon, M. C.: Induction of Ovarian Tumors in Mice by X-Rays, Cancer Research, **7**: 241-245, 1947.
24. Li, M. H., and Gardner, W. U.: Further Studies on the Pathogenesis of Ovarian Tumors in Mice, Cancer Research **9**: 35-41, 1949.
25. Liebow, A. A., Warren, S., and DeCoursey, E.: Pathology of Atomic Bomb Casualties, Am. J. Path. **25**: 853-1027, 1949.
26. Joël, C. A., and von Wattenwyl, H.: Veränderungen in der Hypophyse nach Röntgenbestrahlung des Hodens, Strahlentherapie **71**: 139-150, 1942.

THE PROTECTIVE EFFECT OF SHIELDED ECTOPIC BONE MARROW AGAINST TOTAL BODY X-RADIATION*

J. B. Storer, M.D., C. C. Lushbaugh, M.D., and J. E. Furchner, M.S.
Los Alamos, N. M.

IT HAS been shown that lead shielding of various organs or portions of the body increases survival in experimental animals exposed to lethal doses of x-radiation.[1-3] Shielding of such organs as the spleen or intestine has been accomplished by exteriorizing the organ and enclosing it in lead.[2] A protective effect of shielding bone marrow has been inferred from experiments in which bony structures have been shielded.[1, 3] Until now it has not been shown conclusively that other tissues, such as muscle, connective tissue, primitive reticulum, and bone, which are unavoidably shielded in these experiments, do not contribute to the protective effect, or that this protective effect depends on well-developed hematopoietic tissue.

The mechanism by which this protective effect of shielding is mediated is not known. It has been shown that animals irradiated with their spleens shielded differ from the irradiated controls in the following ways: (a) the hemoglobin values, red blood counts, and hematocrits are not depressed in the shielded animals,[4] (b) the white blood count does not fall to as low levels and returns to normal more rapidly,[4] (c) the iron uptake by red cells is not so severely depressed,[5] and (d) there is a uniform rapid regeneration of bone marrow.[2, 4] A "humoral factor" released by the shielded spleen has been suggested to explain these effects.[2] Lorenz and associates,[6] however, in experiments in mice, transplanted normal bone marrow into irradiated animals and found (a) increased survival, and (b) foci of extramedullary hematopoiesis in the liver. The presence of these foci of cells appears to support a second hypothesis of a "seeding" of normal hematopoietic cells which then proliferate and produce the protective effect. Another possible explanation is based on the belief that the reticulum of fat is capable of differentiating into hematopoietic tissue. The effect of shielding this reticulum on survival of irradiated animals is not known. The principal difficulty in testing this possibility has been in finding a suitable method of stimulating differentiation of normal reticulum into marrow. Huggins and Blocksom[7] showed that if the skinned tail of a rat is placed within the abdominal cavity, red bone marrow will develop in it, whereas normally it contains only fatty marrow. This differentiation is thought to be due to the increased temperature of the tail within the abdominal cavity.

Using Huggins' and Blocksom's technique for producing ectopic marrow in the tails of rats, the survival of rats exposed to total body x-radiation was studied by (a) shielding ectopic tail marrow, (b) shielding normal fatty tail marrow,

From the Los Alamos Scientific Laboratory, University of California.
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and (c) first shielding the fatty tail marrow reticulum and then attempting to produce hematopoiesis in it by abdominal implantation. Determinations of the comparative changes in hemoglobin and white-cell values, in red-cell iron uptake, and in radiosensitive organs in irradiated rats with and without shielded ectopic tail marrow were also made in order to determine whether changes occurred which would support a "humoral" or "seeding" effect as an explanation of the protection obtained by shielding.

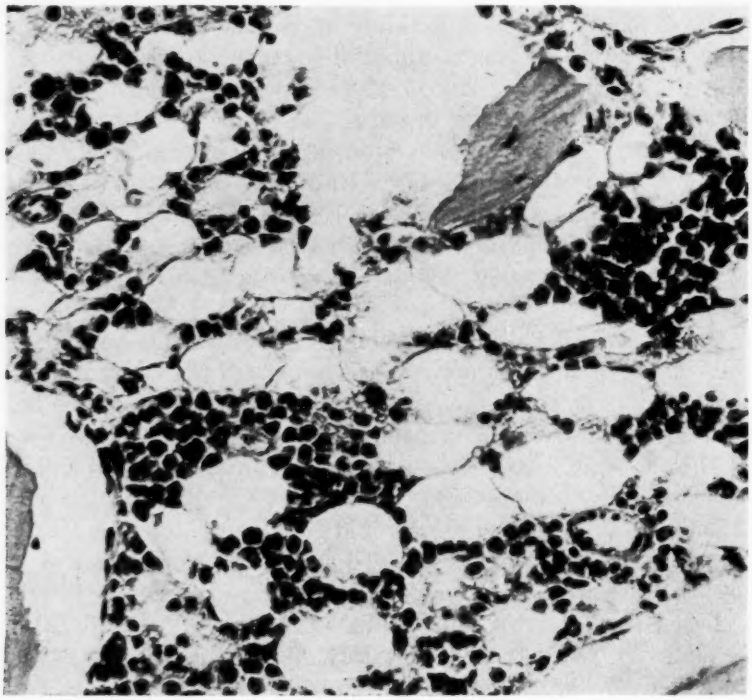

Fig. 1.—Appearance of the marrow cavity of a rat's tail four months after implantation into the abdominal cavity.

MATERIALS AND METHODS

A total of 120 male Sprague-Dawley rats was used. These rats were approximately 5 weeks of age at the time of insertion of the distal portion of the stripped tail into the peritoneal cavity. The radiation experiments were performed four months later, when ectopic marrow was well established. At the time of irradiation the animals were 21 weeks old, so that for the most part survival was studied on adult animals. An exception was the experiment in which the tails of 5-week-old rats were irradiated with the fatty tail marrow shielded and then the tail inserted into the abdomen. All experimental animals were housed five or six to a cage and given water and Purina Laboratory Chow ad libitum prior to and following surgical treatment and x-radiation.

Ectopic marrow formation in the tail was induced in the following way. Five-week-old rats were anesthetized with ether, and the distal two-thirds of the tail was skinned. The skinned portion was inserted into the abdomen through a low right-flank incision and sutured in place by a row of interrupted steel sutures through the skin of the flank and the margin of the skin left on the tail. No attempt was made to close the peritoneal or muscle layers.

No wound disruptions were encountered. Healing was uneventful, and the abdominal and tail skin healed together well. Bleeding from the tail was controlled at the time of operation by tying a rubber band securely around the base of the tail as a tourniquet. Four months later a moderate amount of hematopoietic tissue was found to be present in the marrow cavity of the tail (Fig. 1). This occurrence of hematopoietic tissue in the marrow cavity is in striking contrast to the marrow cavity of the normal tail which contains only fat and reticulum (Fig. 2).

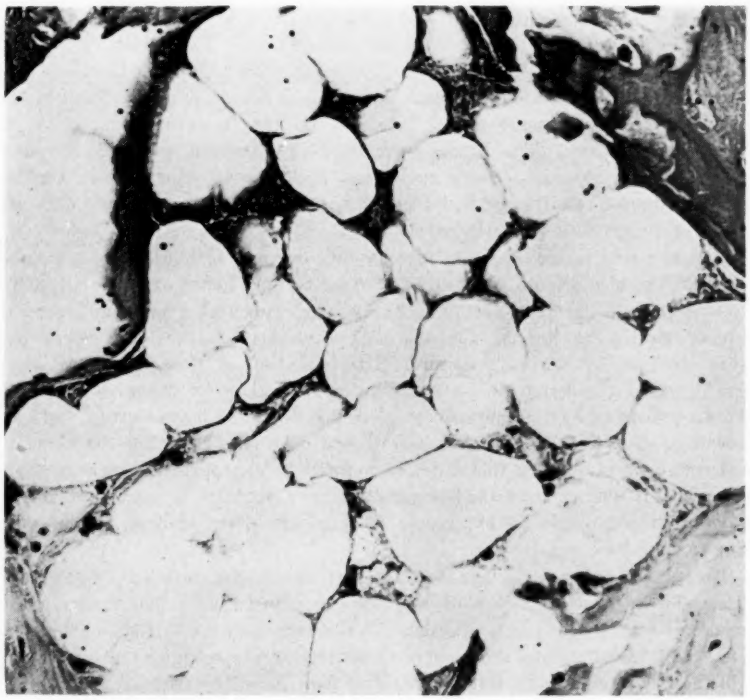

Fig. 2.—Appearance of the marrow cavity of a normal rat tail.

The effect of shielding ectopic red marrow on survival was investigated as follows: seventy-five rats having the distal two-thirds of their tails in their abdominal cavity for the previous four months were used. The tails were exteriorized under pentobarbital anesthesia by making a circular incision through the skin around the tail and dissecting it free from the adherent abdominal muscles. Adhesions between the tail and omentum were commonly encountered, but these were easily lysed. Occasionally the tip of the tail was found to be adherent to the liver capsule. In these cases the tip was cut off rather than dissected free, since attempts at dissection usually resulted in a tearing of the hepatic capsule which then led to fatal hemorrhage. The tail was found to have a fairly abundant collateral circulation with the abdominal wall. It was also found invariably to be ankylosed. Following exteriorization the tail was wrapped in a gauze moistened with saline, shielded or not, and the rat was exposed to total body x-radiation. Shielding was performed in thirty-eight of the seventy-five rats by placing the whole tail between a one-half inch lead shield. The rats were irradiated in pairs, one with the tail shielded and one without. The x-rays were delivered by a Picker Industrial Model machine using the following factors: inherent filtration equivalent to 2 mm. Al with 1 mm. Al and ½ mm. Cu added, 250 K.V.P., 15 Ma. 55 cm. tube specimen distance, 75 r per minute. Radiation dosages were measured in air with a 100 r Victoreen ionization chamber. During irradiation the rats were lying on a one-quarter inch sheet of Lucite suspended in air

by a specially constructed wood frame. Six hundred fifty air roentgens were delivered during each exposure. Following irradiation the shielded and unshielded tails were reinserted in the peritoneal cavity and the wounds were closed with continuous steel sutures. At this time it was found necessary to suture through the entire thickness of the abdominal wall rather than just through the skin to prevent herniation. To determine the effect on survival of shielding the tails of normal rats, forty adult rats were irradiated with 650 r, twenty with the tail shielded by one-half inch of lead. Following these procedures the rats were housed as previously described, and the mortality rate determined.

Blood counts following exposure to 650 r were done on a second group of fourteen rats irradiated with or without shielding of ectopic tail marrow. The operative and irradiation techniques were the same as described above. Two days prior to, and at intervals of two days following, irradiation total white-cell counts, hemoglobin determinations (Sahli method), and differential white-cell counts were done. At intervals rats were sacrificed from these two groups for histologic study. The tissues were fixed in Formalin, sectioned in paraffin, and stained with hematoxylin-eosin. Since there was spontaneous mortality in the unshielded group in addition to the attrition of serial sacrifice, no blood count data were obtained on this group beyond eight days following irradiation.

Iron^{59} uptake was investigated on sixteen rats exposed to 500 r, eight with and eight without shielding of the ectopic tail marrow. Twenty-four hours after irradiation a tracer dose of Fe^{59} was injected into each of the two experimental groups and into eight unirradiated rats serving as normal controls. At intervals of three and seven days blood samples were obtained by cardiac puncture. Determination of red-cell radioactivity was obtained by subtracting the serum radioactivity value from that for the whole blood. Activity measurements were made by the method described by Berlin and associates,[8] and calculations of the percentage of activity in red cells were based on their figures for the blood volume of rats. Blood counts were done on this series of animals at appropriate intervals. Spontaneous mortality, accidental deaths from cardiac puncture, and sacrifice in unsuccessful attempts to obtain radioautographs made it impossible to prolong blood studies to the point where recovery to normal values might occur.

The effect of shielding reticulum capable of differentiation into hematopoietic tissue was studied also. Twenty rats 5 weeks of age were irradiated with 500 r, ten with the tail shielded as described. Following irradiation the ten rats with the tail shielded were operated upon and the tail inserted into the peritoneal cavity by the technique previously described. Mortality in the two groups was followed for thirty days.

RESULTS

Lead shielding of the small amount of ectopic bone marrow formed in the rat's tail by abdominal implantation increased survival following total body x-radiation. Sixty-eight per cent of the rats so treated survived 650 r total body radiation, as opposed to 32 per cent of those receiving the same operative treatment but irradiated without shielding. Analysis of these data by the chi square method gives a p value of < 0.01. These data are shown in Table I. Normal rats having only fatty marrow in their tails showed no increase in survival (Table I) when irradiated with their tails shielded. The survival in the two groups of twenty normal adult rats irradiated with and without their tails shielded was 45 per cent and 55 per cent, respectively. A p value of < 0.5 is obtained for this difference by the chi square method.

Uptake of Fe^{59} by the red cells of rats irradiated with 500 r with and without ectopic marrow shielding and of normal unirradiated rats is shown in Fig. 3. Each point on the graph represents the average value for five rats. Both the irradiated groups showed pronounced depression of uptake at the two intervals

at which blood samples were withdrawn. At three days there was no significant difference between the two groups. On the seventh day the rats irradiated with the tail shielded showed a slightly greater percentage of iron uptake by the red cells in their cells than did those irradiated with the tail unshielded.

The changes in the white count and hemoglobin levels of rats irradiated with 650 and 500 r are shown in Figs. 4 and 5. Data on the relative rate of recovery of these values for the two groups were not obtained since spontaneous mortality prevented a continuation of the study.

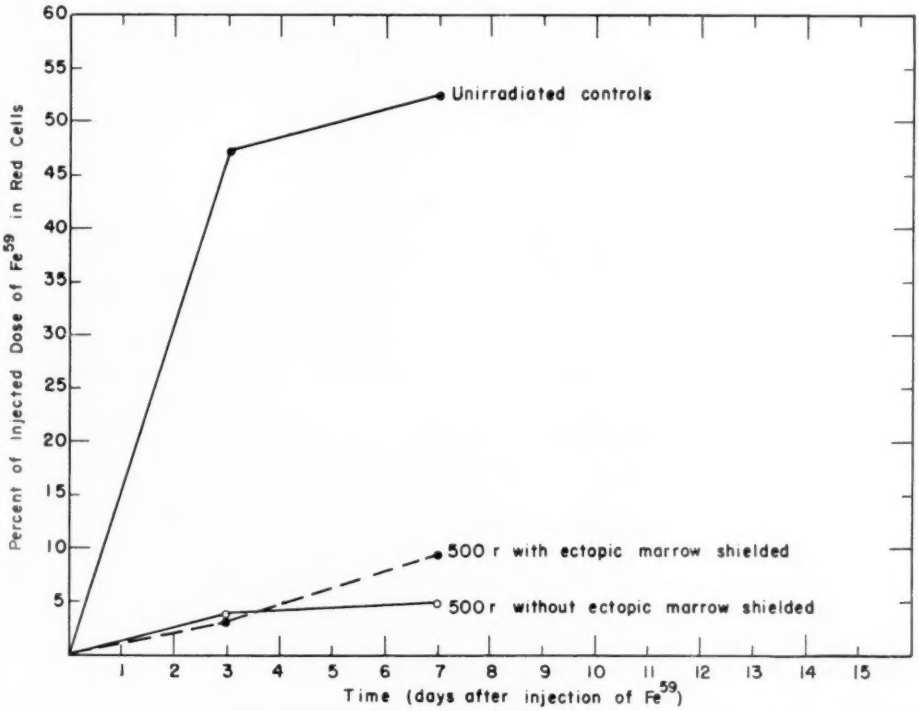

Fig. 3.—Per cent of Fe^{59} appearing in red cells at intervals after a single intravenous injection, in control rats, in rats subjected to 500 r total body x-ray, and in rats irradiated with 500 r with shielding of ectopic tail marrow.

Histologic studies showed that four months following abdominal implantation there was a moderate amount of marrow present in the tail in contrast with normal tails which contained only fatty marrow. One day after irradiation there were the usual degenerative changes in the thymus and lymphoid tissue. The unshielded tails and femoral marrows of both groups showed "hemorrhagic" marrow with pronounced destruction of cells. The shielded marrow showed engorgement of venous sinuses but not cell destruction. There was no extramedullary hematopoiesis in kidney or liver. There was considerable red-cell debris phagocytized in the spleen. Two days after irradiation the degenerative changes had progressed, but were otherwise similar to those seen at one day. At seven days after exposure there was still no evidence of hyperplasia of the

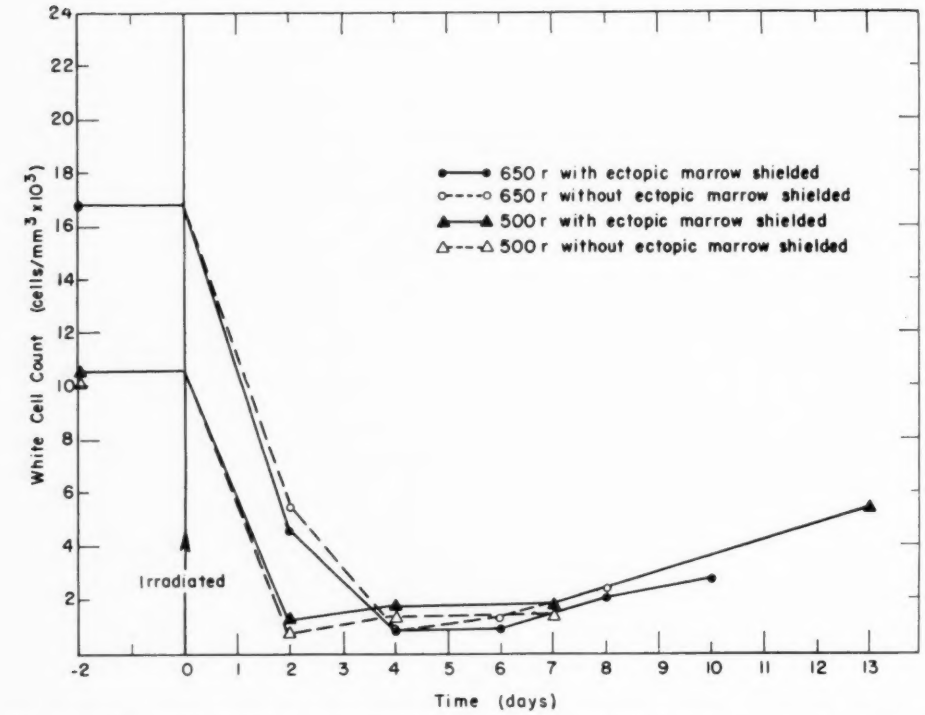
650 r with ectopic marrow shielded
650 r without ectopic marrow shielded
500 r with ectopic marrow shielded
500 r without ectopic marrow shielded
White Cell Count (cells/mm³ x10³)
Irradiated
Time (days)

Fig. 4.—White blood counts in rats irradiated with 500 r and 650 r with or without shielding of ectopic tail marrow.

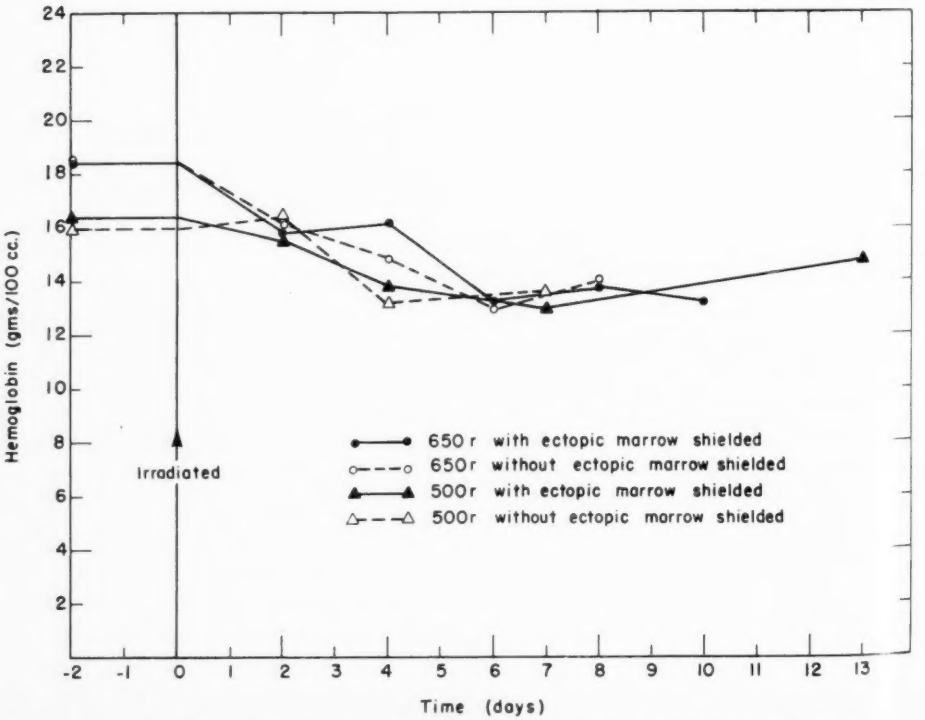

Fig. 5.—Hemoglobin values (Sahli) in rats irradiated with 500 r and 650 r with or without shielding of ectopic tail marrow.

TABLE I. THIRTY-DAY SURVIVAL IN ADULT RATS IRRADIATED WITH 650 R TOTAL BODY X-RADIATION WITH AND WITHOUT SHIELDING OF RED OR FATTY MARROW IN THE TAIL

NO. RATS	TREATMENT	30-DAY SURVIVAL	PER CENT SURVIVAL	SIGNIFICANCE OF DIFFERENCE
38	Irradiated with shielding of ectopic tail marrow	26	68	
37	Irradiated without shielding of ectopic tail marrow	12	32	$p = 0.01$
20	Irradiated with shielding of normal tail	9	45	
20	Irradiated without shielding of normal tail	11	55	$p = 0.5$

marrow either in the tail or the femur of animals that had the tail shielded during radiation. The debris in the marrow was cleaned up by this time, and an extreme loss of cellularity was apparent. No extramedullary hematopoiesis was seen in any of the sections. The spleens from both groups were atrophic, and contained considerable hemosiderin.

The effect on survival of shielding the fatty marrow reticulum during irradiation, and then implanting the tail in the abdomen, is summarized in Table II. None of the ten young rats so treated survived for thirty days, as opposed to five survivors of the ten unshielded, unoperated, irradiated controls.

TABLE II. EFFECT ON SURVIVAL OF YOUNG RATS OF SHIELDING RETICULUM IN FATTY TAIL MARROW DURING IRRADIATION WITH 500 R FOLLOWED BY IMPLANTATION OF THE TAIL INTO THE ABDOMEN

NO. RATS	TREATMENT	30-DAY SURVIVAL	PER CENT SURVIVAL
10	Irradiated with tail shielded. Tail implanted in abdomen following irradiation	0	0
10	Irradiated without shielding. No operation	5	50

DISCUSSION

Survival of rats exposed to 650 r of x-radiation was significantly increased when ectopic marrow in the tail was shielded during irradiation. Since survival of rats irradiated with shielding of the normal tail, containing no red marrow, was not increased, it is apparent that the beneficial effect of shielding bony structures is due to protection of the hematopoietic tissue per se rather than to protection of the unavoidably shielded bone, cartilage, fat, reticulum, and fibrous tissue.

The characteristic differences reportedly found between animals irradiated with and without spleen shielding were not found between rats irradiated with and without shielding of ectopic bone marrow. The hematologic changes for the first eight days following irradiation were identical for the two groups. Whether or not final recovery of the blood picture to normal values was hastened in the shielded group could not be determined, since spontaneous mortality, serial sacrifice, and accidental deaths from cardiac puncture left no rats living in the unshielded group at ten and thirteen days for the animals receiving 650 r and 500 r, respectively. There was a suggestion of a more rapid

recovery of erythropoiesis in the shielded group in the iron uptake study. This more rapid recovery, however, was not confirmed histologically. There was no apparent difference in histologic changes between the two groups in any of the tissues studied except in the tail marrow. In the shielded animals this marrow showed no evidence of degeneration, but neither did it show hyperplasia.

It seems likely that the reason for the lack of any significant differences in the blood counts, iron uptake by red cells, and histology of the two groups was that the amount of marrow shielded was too small to produce measurable differences in the various factors which were studied. The marrow cavity of the tail was found to be extremely small and, while a moderate amount of marrow was present, it did not approach the amount found per unit area in other marrow-containing bones such as the femur. If the minuteness of the amount of marrow shielded is the true explanation of the lack of differences observed, it must be concluded that survival remains the most sensitive criterion in evaluating prophylactic measures.

It also appears that only minute amounts of normal marrow are necessary to increase survival following irradiation. The lack of evidence of an increased rate of regeneration of the unshielded marrow indicates that such regeneration is not necessarily related to increased survival. This fact is also apparent from the data of Jacobson and associates on the effect of shielding the head or exteriorized intestine of mice exposed to 1,025 r of x-radiation.[2] At eight days following irradiation the regeneration of marrow in mice exposed with their intestines shielded was "nearly complete," while marrow recovery in the head-shielded group was "only partial." Despite these differences in regeneration of the marrow, the percentage survival in the two groups was 26.6 per cent and 27.7 per cent, respectively.

The exact mechanism by which survival is affected through the presence of undamaged hematopoietic tissue is not known. In view of the lack of findings in the present study to support either the so-called humoral or seeding theories, it appears necessary to consider a third possible explanation, namely, that the undamaged marrow or spleen serves as a focus for sufficient hematopoiesis to support life until the irradiated but relatively radioresistant marrow reticulum can reconstitute hematopoiesis elsewhere.

The presence of growing ectopic hematopoietic foci following intravenous injection of normal marrow into irradiated mice[6] cannot be considered as evidence in favor of the seeding theory. It would, however, seem to support the observation that only minute amounts of normal viable marrow are needed to enhance survival in irradiated animals. The presence of ectopic marrow following an intravenous injection of marrow is only the expected result of such a procedure. If marrow regeneration depended on seeding by way of the blood stream, ectopic hematopoiesis would be expected in many organs other than the marrow spaces. Such ectopic foci were not found in the present study, although foci in the liver of spleen-shielded mice have been reported.[4]

The humoral theory for explaining increased survival in spleen-shielded animals is based primarily on the fact that regeneration of the irradiated

marrow is hastened in these animals. Since an increased rate of regeneration is not essential for producing increased survival (*vide supra*) it seems likely that the critical factor in determining survival in an animal is whether or not there are sufficient undamaged circulating white cells to maintain the minimal defense mechanisms required to sustain life until regeneration of marrow occurs. A hastening of marrow regeneration would be advantageous, since it would shorten the period of dangerously low levels of circulating normal white cells, but it is probably not of primary importance.

Circulating undamaged white cells can be provided in irradiated animals in a number of ways. Marrow or spleen transplantation or blood transfusions following irradiation or shielding of hematopoietic tissue during irradiation provide such cells. All these procedures increase survival. It is significant that lysed marrow cells do not increase survival.[9] This fact strongly suggests that intact cells are required. The recent report by Cole and associates[10] on the protective effect of spleen homogenates does not conflict with this idea, since intact cells were present in the material they injected.

Evidence of maintenance of the defense mechanisms during the period following irradiation in partially shielded animals was found in rabbits irradiated with shielding of the spleen or lymphoid tissue of the appendix.[11] Higher antibody titers were produced by these animals following the injection of antigen than were found in the irradiated controls. It has been shown also that strain-specific resistance to leukemic transplants is higher in CF-1 mice irradiated with spleen or head shielding than in nonshielded controls irradiated with the same dosage of x-radiation.[12]

The observation that surgical removal of the shielded spleen following irradiation does not prevent increased survival in mice[2] does not conflict with the thesis that protection is mediated by a relatively small number of circulating normal white cells. One to six hours following irradiation the shielded spleen can be removed without adversely affecting survival.[2] The spleen of rodents normally contains foci of lymphopoiesis and myelopoiesis. Within one to six hours following irradiation the shielded spleen must release a sufficiently large number of normal white cells into the general circulation to insure survival. This premise is supported by the fact that various types of acute stress have been shown to produce a rapid increase in circulating white cells. These increases may occur in a matter of minutes rather than hours. (For a review of the literature see Michael.[13]) Presumably the leukocytosis induced by stress is due either to release of large numbers of cells by hematopoietic centers or to contraction of the spleen with a "squeezing out" of cells into the circulation. The initial rise in heterophil levels produced in rabbits[14] and rats[15] by total body x-radiation indicates that this radiation serves as a suitable stress for increasing the white count initially.

It is not necessary to postulate a humoral factor as being responsible for the increased rate of regeneration of the marrow of spleen-shielded animals. It is well known that the rate of repair of damaged tissues depends on the general health and metabolic state of the animal. For example, the healing

of surgical wounds is greatly impaired in protein-depleted rats.[15] The general health of animals irradiated with their spleens shielded is obviously better objectively than that of the irradiated controls. It is not surprising, then, that the reparative process in the marrow of the shielded mice proceeds more rapidly. The same conclusion can be reached from a different point of view. Animals irradiated with low doses of radiation show a more rapid recovery of the bone marrow than do animals irradiated with large doses.[17] No doubt the marrow is more severely damaged at higher doses but in the repair process it is not a simple matter of a return to normal of the damaged mature cells but of their replacement by new cells. This replacement occurs faster in animals receiving the lower doses, presumably partly because the general health of the animals is better than that of the animals exposed to higher doses. An analogous situation exists clinically where persons severely ill may show a "toxic" depression of marrow function in the absence of specific damage to the marrow per se, while other patients less severely stricken with the same disease may show an extensive leukocytosis.

It seems likely that the rats used in the present study did not show an increased rate of marrow regeneration because the number of normal cells circulating was barely sufficient to sustain life, and their general health was only slightly better than that of the irradiated controls. In experiments in which animals are irradiated with their spleens shielded it is probable that more hematopoietic tissue is being shielded than the minimum necessary to insure survival at the radiation doses used. At radiation dosages of 1,025 r (the LD-50 for spleen-shielded mice), no anemia and only a transient leukopenia occur.[4] That this degree of maintenance of hematopoietic function is not necessary to insure survival has been shown by the present study. The shielding of excessive amounts of hematopoietic tissue probably maintains the animals in far better general health than the minimum necessary for survival. As a result of better general health it would be anticipated that regeneration of radiation-damaged tissues such as the bone marrow would be hastened.

It would appear that if more hematopoietic tissue is being shielded in spleen-shielded mice than is necessary to maintain the minimum defense mechanisms required to ensure survival, no mice would die at doses of 1,025 r, but such is not the case. However, on the basis of work by Petersen and Dubois,[18] it appears that the reason for death in these animals may not be due to lack of sufficient hematopoiesis to support life but to irreparable damage to some tissue or tissues other than those engaged in hematopoiesis. In these experiments with rats, 1,250 r delivered to the abdomen were lethal, even though the relatively vast amounts of marrow present in the head, thorax, and extremities were protected during delivery of the radiation. This fact suggests that some second critical tissue (the hematopoietic and lyhphopoietic systems being the first) is so severely damaged by this dose of radiation that maintenance of normal or nearly normal defense mechanisms is insufficient to insure survival. A similar hypothesis has been advanced by Jacobson and associates to explain mortality in mice irradiated with 1,000 r with spleen shielding.[4]

The lack of effect on survival of abdominal implantation of the normal shielded tail following irradiation is not surprising. In view of the above considerations, it would be anticipated that marrow formation would occur even more slowly in irradiated rats than in normal rats. Since a long latent period for this transformation was found in normal rats, it is apparent that the process occurs at too slow a rate to influence survival following acute radiation exposures.

The present study does not elucidate the mechanism by which shielding of hematopoietic tissue exerts its protective effect. Considerably more work must be done before it can be stated with any certainty which, if any, of the possible mechanisms discussed is responsible for increased survival.

SUMMARY AND CONCLUSIONS

Lead shielding of small amounts of ectopic marrow induced in the tails of rats by abdominal implantation increased survival of rats exposed to 650 r of total body x-radiation. Shielding of normal tails containing fatty marrow did not increase survival, indicating that marrow cells per se rather than other tissues unavoidably shielded are responsible for increased survival in bone-shielding experiments. Implantation of shielded normal tail into the abdomen following irradiation did not increase survival of rats because of the slowness with which ectopic marrow develops from fatty reticulum. There was no significant difference in the changes in hemoglobin values, white blood counts, or radioiron uptake of rats irradiated with or without shielding of ectopic marrow. No histologic evidence of increased regeneration of bone marrow or of ectopic hematopoiesis was found in the shielded rats. The fact that survival was increased in the absence of evidence of increased marrow regeneration indicates that survival in animals irradiated with shielding of hematopoietic tissue does not depend on a more rapid rate of recovery of the unshielded bone marrow.

REFERENCES

1. Gershon-Cohen, J., Hermel, M. B., and Griffith, J. Q., Jr.: The Value of Small Lead Shields Against the Injurious Effect of Total-Body Irradiation, Science **114**: 157, 1951.
2. Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J., and Eldredge, J. H.: Further Studies on Recovery from Radiation Injury, J. Lab. & Clin. Med. **37**: 683, 1951.
3. Allen, J. G.: The Beneficial Effects of Head Shielding in 20 Dogs Exposed to 450 r Total-Body X-radiation, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, February, March, April, 1951, ed. by A. M. Brues. pp. 60-61.
4. Jacobson, L. O., Simmons, E. L., Marks, E. K., Robson, M. J., Bethard, W. F., and Gaston, E. O.: The Role of the Spleen in Radiation Injury and Recovery, J. Lab. & Clin. Med. **35**: 746, 1950.
5. Huff, R. L., Bethard, W. F., Garcia, J. F., Roberts, B. M., Jacobson, L. O., and Lawrence, J. H.: Tracer Iron Distribution Studies in Irradiated Rats with Lead-Shielded Spleens, J. Lab. & Clin. Med. **36**: 40, 1950.
6. Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E.: Modification of Irradiation Injury in Mice and Guinea Pigs by Bone Marrow Injections, J. Nat. Cancer Inst. **12**: 197, 1951.
7. Huggins, C., and Blocksom, B. H.: Changes in Outlying Bone Marrow Accompanying a Local Increase of Temperature within Physiological Limits, J. Exper. Med. **64**: 253, 1936.

8. Berlin, N. I., Huff, R. L., Van Dyke, D. C., and Hennessey, T. G.: The Blood Volume of the Adult Rat as Determined by Fe^{59} and P^{32} Labelled Red Cells, Proc. Soc. Exper. Biol. & Med. **71**: 176, 1949.
9. Straube, R. L., Patt, H. M., and Jackson, E. M.: Progress Report: Relative Effectiveness of Marrow Suspensions Prepared from Normal and Estrogen-Treated Mice on Radiation Toxicity, Quarterly Report, Argonne National Laboratory, May, June, July, 1951, ed. by A. M. Brues, pp. 57-59.
10. Cole, L. J., Fishler, M. C., Ellis, M. E., and Bond, V. P.: Protection of Mice Against X-Irradiation by Spleen Homogenates Administered after Exposure, USNRDL-339, Jan. 15, 1952.
11. Jacobson, L. O., Robson, M. J., and Marks, E. K.: The Effect of X-radiation on Antibody Formation, Proc. Soc. Exper. Biol. & Med. **75**: 145, 1950.
12. Storer, J. B.: Unpublished data, 1951.
13. Michael, S. T.: Adaptation to Brief Stress. The Blood Level of Leucocytes and Adrenal Function in Epilepsy, Electrically Induced Convulsions, and after Injection of Epinephrine. A Review and Interpretation, Yale J. Biol. & Med. **22**: 71, 1950.
14. Jacobson, L. O., Marks, E. K., and Lorenz, E.: The Hematological Effects of Ionizing Radiations, Radiol. **52**: 371, 1949.
15. Ingram, M., and Mason, W. B.: The Effects of Acute Exposure to Roentgen Radiation on the Peripheral Blood of Experimental Animals, Univ. of Rochester Atomic Energy Project, Report UR-122, 1950.
16. Kobak, H. W., Benditt, E. P., Wissler, R. W., and Steffee, C. H.: The Relation of Protein Deficiency to Experimental Wound Healing, Surg., Gynec. & Obst. **85**: 751, 1947.
17. Bloom, W. A.: Histopathology of Irradiation from External and Internal Sources, ed., W. A. Bloom, NNES, Div. IV, 22 I, ed. 1, New York, 1948, McGraw-Hill Book Co.
18. Petersen, D. F., and Dubois, K. P.: Effect of Partial Shielding on the Mortality of X-Irradiated Rats, U.S.A.F. Radiation Laboratory Quarterly Report No. 2, January, 1952, pp. 22-25.

PLASMA ANTITHROMBIN IN THROMBOSIS, HEMORRHAGE, AND LIVER DISEASE

WILLIAM H. GRIFFITH, M.D.*
LOS ANGELES, CALIF.

CHANGES in the antithrombin activity of plasma appear to be of some significance in a number of forms of disease. Interest in this subject has been stimulated by reports of Kay and associates,[1] which indicate that a deficiency of antithrombin may be a factor in the development of thrombosis, although several other workers[2-4] have failed to find a correlation between antithrombin and thrombosis. Recently, Innerfield, Angrist, and Benjamin[5] have reported that a marked increase in plasma antithrombin occurs in nearly all cases of acute pancreatitis, and in obstructive jaundice due to carcinoma of the pancreas. They believe that determinations of plasma antithrombin can be an important aid in the diagnosis of these conditions.

Beginning with Howell,[6] a considerable number of workers have described methods for the quantitative determination of antithrombin. The author has been using a method which is a modification of the one devised by Astrup,[7] and which is suitable for clinical use.

METHOD AND MATERIAL

All methods for determining antithrombin involve the addition of a known amount of thrombin to a known amount of plasma (or serum) and the assay of the thrombin remaining after suitable reaction periods. In order to eliminate loss of thrombin through adsorption by fibrin, fibrinogen must first be removed from the plasma, either by heating to 56° C., or by preliminary treatment with a small amount of thrombin. Serum has been used, but there is the objection that it has been exposed to an unknown amount of thrombin in clotting. Klein and Seegers[8] determine the maximum amount of thrombin that can be inactivated by one milliliter of plasma, using a comparatively large amount of thrombin and a reaction period of two hours or more. Other methods which use only a few minutes' incubation time determine only the rate at which thrombin is inactivated under the conditions of the test. The rate of this reaction appears to be a satisfactory index of antithrombin activity, or at least a practical means for comparing the activity of different plasmas.

If relatively small quantities of thrombin are used, a system can be set up which will determine the time required for about 80 per cent inactivation of the added thrombin. Plasma having a deficiency of antithrombin will require a longer incubation period for 80 per cent inactivation than normal plasma. Thus it is possible to measure antithrombin in a system in which only the incubation time is varied. The result can be expressed as units of thrombin inactivated per minute for a given set of conditions. Thrombin remaining after the period of incubation is measured by determining the clotting time of a known amount of added fibrinogen.

The thrombin solution used contains twenty-five units per milliliter. Commercially available bovine thrombin (Topical Thrombin, Parke, Davis & Company) proved to be satisfactory in this dilution.

Comparisons were made with highly purified thrombin which was kindly supplied by Dr. Walter H. Seegers. Similar results were obtained when solutions of the same potency were used. The technique described by Ware and Seegers[9] was used to compare the

From the Department of Internal Medicine of the Hollywood Presbyterian Hospital, Los Angeles, Calif.
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*Present address: 6634 Sunset Blvd., Los Angeles 28, Calif.

strength of different thrombin solutions. Specially assayed thrombin was provided by Eugene C. Loomis of Parke, Davis & Company to be used as the standard of potency. The commercial product was used for the test itself. It is believed to be sufficiently constant to be used in routine work.

TECHNIQUE

Five units of thrombin (0.2 ml. of a solution containing 25 units per milliliter) are added to 0.2 ml. of defibrinated plasma. After an interval of exactly three minutes, 0.2 ml. of fibrinogen solution is added, and the time for clotting is determined. The amount of thrombin remaining is indicated by the clotting time of the added fibrinogen, as shown in Table I. From this figure, the number of units of thrombin, inactivated per minute by 1 ml. of plasma, is easily calculated. If more than 1.2 units of thrombin, or less than 0.7 units are left the procedure is repeated, using a longer or shorter incubation time.

MATERIALS

Defibrinated Oxalate Plasma.—4.5 ml. of patient's blood is added to 0.5 ml. of 0.1M sodium oxalate. Centrifuge and separate plasma. Remove fibrinogen by heating to exactly 56° C. for thirty seconds and cooling quickly. Centrifuge to eliminate precipitated fibrinogen.

Thrombin Solution.—A stock solution containing 1,000 units per milliliter is prepared by dissolving the contents of one 5,000 unit vial of Bovine Topical Thrombin (Parke, Davis & Company) in 50 per cent glycerol and making up to exactly 5.0 milliliter. This solution is stable at −5° C. for several weeks. It can be divided into 1 ml. portions and stored in small tubes. Working solutions are made each day in distilled water without buffer. Usually 0.2 ml. of stock solution is diluted to 8.0 ml. to provide a solution containing 25 units per milliliter. A further dilution is made to provide a solution having 5.0 units per milliliter to use in a control test.

Fibrinogen Solution.—The Armour Laboratories' Bovine Fibrinogen, 3 per cent solution (including citrate) in saline with imidazole buffer.[9] Fibrinogen solution is freshly prepared. Incubate thirty minutes at 37° C. to dissolve. Solution is stable at room temperature for an hour or more.

Buffered saline—0.9 per cent NaCl	47.5 ml.
imidazole buffer solution	2.5 ml.

0.3 Gm. portions of fibrinogen powder can be stored in deep freeze to be dissolved as needed. Stronger or weaker solutions of fibrinogen can be used if necessary to bring the clotting time for one unit of thrombin within the range covered by Table I.

PROCEDURE

Two-tenths milliliter patient's plasma (defibrinated oxalate plasma) added rapidly to 0.2 ml. thrombin solution—25 units per milliliter. Start stop watch. Incubate at about 37° C. Minor variations in temperature appear to have little influence on the result. In exactly three minutes, add quickly 0.2 ml. of fibrinogen solution. Start stop watch—determine time for clotting of added fibrinogen, by noting first definite fibrin formation. (Use strong side light and dark background. The timing of both steps can be done with one watch, by stopping the watch two seconds early in the first step, and allowing a two-second interval to add the fibrinogen.)

If clotting time does not fall within range covered in table, repeat test, using a longer incubation time if clotting time is short, shorter incubation time if clotting time is long. Read the quantity of thrombin remaining, by referring to Table I.

Express result in terms of units of thrombin inactivated per minute, by 1 ml. of plasma. (No allowance for dilution with oxalate.)

$$\text{Units inactivated per minute} = \frac{5 \times (5 - u)}{T}$$

u = units remaining in tube
T = incubation time in minutes

TABLE I. CONTROL TEST—CLOTTING TIME FOR VARIOUS AMOUNTS OF THROMBIN, UNDER THE CONDITIONS OF THE INDIVIDUAL TEST

CLOTTING TIME (SECONDS) WITH ONE UNIT THROMBIN	13	14	15	16	17
Clotting time of unknown	Units thrombin in unknown				
11	1.2				
12	1.1	1.2			
13	1.0	1.1	1.2		
14	0.92	1.0	1.1	1.1	
15	0.85	0.93	1.0	1.07	1.15
16	0.78	0.87	0.94	1.0	1.07
17		0.81	0.87	0.93	1.00
18			0.81	0.86	0.92
19				0.82	0.87
20					0.83

Procedure—0.2 ml. patient's plasma, defibrinated.
0.2 ml. fibrinogen solution.
Add 0.2 ml. thrombin solution 5 units per milliliter.
Determine time for clotting. (No incubation period.)

This is the time for clotting with one unit of thrombin under conditions of the test. Compensates for variations in activity of fibrinogen solution, and for minor variations in effectiveness of thrombin, which can occur with different plasmas.

Clotting time for one unit will determine which column in Table to use for final calculation.

TABLE II. NORMAL VALUES OF ANTITHROMBIN, AS UNITS OF THROMBIN INACTIVATED PER MINUTE BY 1 ML. OF DEFIBRINATED PLASMA, WITH 25 UNITS OF THROMBIN ADDED

RANGE	NO. OF CASES
6.0 to 6.9 u/min.	18
7.0 to 7.9	5
8.0 to 8.9	3
9.0 to 10.0	2
Average value for 28 presumably normal adults	7.1 u/min.
Lowest figure obtained in this group	6.0
Highest figure	10.0

TABLE III. ANTITHROMBIN IN NORMAL SERUM AND PLASMA. ANTITHROMBIN ACTIVITY EXPRESSED IN TERMS OF UNITS OF THROMBIN INACTIVATED PER MINUTE, BY 1 ML. PLASMA OR SERUM, WITH 25.0 UNITS OF THROMBIN ADDED (NO ALLOWANCE FOR DILUTION WITH ANTICOAGULANT)

	UNITS THROMBIN INACTIVATED PER MINUTE
1. Normal oxalate plasma (defibrinated) average value	7.1
2. Normal serum, average value	4.9
3. Serum treated with Amberlite Resin IR 100.	6.5
4. Oxalated serum (serum 4 vol. plus 1 vol. 0.1M. sodium oxalate solution)	6.4
5. Al $(OH)_3$ adsorbed plasma. (1 vol. Al $[OH]_3$ cream, 9 vol. blood, no other anticoagulant)	5.0

TABLE IV. ANTITHROMBIN IN PATIENTS WITH THROMBOSIS.
VENOUS THROMBOSIS

CASE NUMBER	DIAGNOSIS	UNITS/MIN. ANTITHROMBIN	REMARKS
1	Recurrent venous thrombosis, legs	6.5	
2	Venous thrombosis, arm	8.0	
3	Venous thrombosis, legs	8.0	Small pulmonary emboli
4	Pulmonary embolism, post-operative	20.0	Plasma icteric, death in 24 hours. No autopsy
5	Pulmonary embolism, post-operative	9.7	Splenectomy following traumatic rupture of spleen
6	Thrombophlebitis, legs	8.6	Fibrinogen 2 × normal, no emboli
	Cerebral Thrombosis		
7	Cerebral thrombosis	8.0	
8	Cerebral thrombosis	10.0	
	Coronary Thrombosis		
9	Coronary thrombosis, acute	8.0	
10	Coronary thrombosis, acute	6.2	
11	Coronary thrombosis, acute	8.2	

Antithrombin as units of thrombin inactivated per minute by 1 ml. plasma, with 25 units thrombin added.
Patients receiving anticoagulant therapy excluded.

A control test is run with each plasma, using one unit of thrombin and no incubation period. The incubation period is eliminated by changing the order of adding the materials.

0.2 ml. defibrinated oxalate plasma
0.2 ml. fibrinogen solution
add
0.2 ml. thrombin solution, 5 units per milliliter (1 unit).

Determine the time for clotting. This is the clotting time equivalent to one unit of thrombin, under the exact conditions of the individual test, and will indicate the appropriate column in Table I to use in the final calculation.

TABLE V. BISHYDROXYCOUMARIN PLASMA

CASE NUMBER	NUMBER OF DAYS OF TREATMENT	PROTHROMBIN PER CENT OF NORMAL	ANTITHROMBIN UNITS/MIN.	DIAGNOSIS
1	4	15	9.2	Post-partum thrombophlebitis
	6	18	12.7	
	7	16	13.0	
	11	30	7.4	
2	1	90	8.0	Coronary thrombosis
	2	60	8.8	
	3	25	10.4	
3	5	45	12.5	Coronary thrombosis
	8	70	13.3	
4	5	34	9.6	Coronary thrombosis
5	6	40	10.0	Coronary thrombosis
6	25	40	13.3	
	35	45	9.6	
7	4	50	10.0	Cerebral thrombosis
8	15	10	7.8	Thrombophlebitis
9	5	50	9.3	Coronary thrombosis
	10	24	10.5	
10	14	15	12.7	Coronary thrombosis
11	15	12	13.5	Coronary thrombosis

Prothrombin expressed as per cent of normal, Quick, one-stage method.

Caution.—In an occasional case a small amount of fibrinogen may remain in the plasma after it has been heated as directed above. This will adsorb thrombin and will result in an erroneously high value for antithrombin. The presence of fibrinogen can be detected by noting the formation of a flocculent precipitate of fibrin when thrombin is added in the first step of the test. It can be removed by heating the plasma again, but overheating is to be avoided.

RESULTS

Table II shows the distribution of values found in normal individuals.

Table III shows a comparison of serum and plasma. Defibrinated oxalate plasma has about 30 per cent greater antithrombin activity as measured by the present method. Part of this difference appears to have some relation to the presence of calcium in serum, since it is less marked if oxalate is added to serum, or if serum is treated with an ion exchange resin as prepared by Stefanini.[10]

TABLE VI. HEMORRHAGIC DISEASE AND MISCELLANEOUS CONDITIONS

CASE NUMBER	DIAGNOSIS	ANTITHROMBIN	REMARKS
1	Leukemia, acute stage	12.5	Active bleeding
2	Hodgkin's disease	9.5	See below
3	Bladder hemorrhage	9.8	See below
4	Bladder hemorrhage	6.6	See below
5	Bladder hemorrhage	13.3	See below
6	Purpura allergic	8.0	Plasma icteric
7	Purpura	6.8	See below
8	Purpura	6.6	See below
9	Purpura	21.2	Obstructive jaundice
10	Purpura	12.0	See below
11	Thrombocytopenic purpura	13.6	Platelets 9,000 Active bleeding
12	Hemophilia	8.2	
13	Hemophilia	7.8	
14	Hemophilia	10.1	
15	Hemophilia	9.2	
16	Multiple myeloma	5.0	Bleeding from gums, fibrinogen increased
17	Multiple myeloma	5.0	No bleeding
18	Adhesive pericarditis	6.6	Edema and ascites. No bleeding
19	Uterine bleeding	8.0	See below
20	Chronic lymphatic leukemia	7.7	No bleeding

1. Leukemia in acute phase with bleeding. Prothrombin 40 per cent.
2. Hodgkin's disease with leukopenia (w.b.c. 1,200) following x-ray therapy. Was in recovery stage from reaction when specimen taken.
3. Bladder hemorrhage—r.b.c. 3,500,000—platelets 210,000—prothrombin 50 per cent (one stage, Quick)
4. Hematuria with no organic lesion. Platelets and prothrombin normal. No anemia.
5. Hematuria and purpuric lesions in skin. Prothrombin 20 per cent of normal (one stage, Quick). r.b.c. 3,400,000—platelets 267,000.
6. Allergic type of purpura. All clotting factors normal.
7. Thrombocytopenic purpura. Prothrombin normal.
8. Thrombocytopenic purpura. Prothrombin normal.
9. Obstructive jaundice with large purpuric areas in skin, three weeks' duration due to common duct disease. Platelet count, prothrombin, Lee White clotting time, and capillary fragility, all normal.
10. Purpura, cause undetermined. Prothrombin and platelets normal.
16. Multiple myeloma, bleeding from gums. Prothrombin and platelets normal. Fibrinogen concentration about 2 × normal.
17. Multiple myeloma, proved on bone marrow study. No bleeding.
19. Excessive uterine bleeding. Receiving x-ray, postoperatively, breast and pelvis. Leukopenia 2,400 and anemia, r.b.c. 2,240,000.

Table IV shows antithrombin activity in patients with thrombosis. A deficiency has not been found in any patient with thrombosis.

Table V shows patients under treatment with bishydroxycoumarin. Some increase in antithrombin was found in most of these cases, as previously reported by Hurn, Barker, and Mann.[11]

Table VI shows patients with hemorrhagic diathesis, and miscellaneous conditions. Antithrombin was elevated in a few patients with abnormal bleeding, although there is no evidence that this was a causative factor. Four patients with hemophilia had normal antithrombin (the opportunity to examine plasma from these patients was afforded by Dr. Arnold G. Ware).

Table VII shows patients with jaundice. A wide range in antithrombin activity was found in this group.

DISCUSSION

An effort has been made to measure antithrombin specifically, as distinguished from the effects of heparin plus co-factor, and the adsorption of thrombin by fibrin. The addition of heparin to plasma, either in vivo or in vitro, will produce an apparent increase in antithrombin as shown by this technique. It will also prolong the clotting time in the control test, which is made with no incubation period. These effects of heparin can be neutralized by a small amount of protamine. Antithrombin itself does not appear to be inhibited by protamine.

In the small series of cases studied, the most marked changes in plasma antithrombin were noted in patients with jaundice. No definite correlation with

TABLE VII. JAUNDICE CASES

CASE NUMBER	DIAGNOSIS	ANTITHROMBIN	REMARKS
	a. Obstructive		
1	Stenosis common duct	21.2	Jaundice 4-week duration. Platelets, prothrombin, Lee White, capillary fragility, normal
2	Carcinoma of the lungs	12.7	Metastasis to liver
3	Carcinoma pancreas	13.3	Surgical exploration
4	Inflammatory mass	13.3	
5	Carcinoma pancreas	23.0	High icterus index, pos. direct van den Bergh. X-ray evidence of tumor
	b. Nonobstructive		
1	Catarrhal jaundice	5.5	Recovery, 6 weeks
2	Hepatitis	2.5	Prothrombin 25 per cent, died
3	Hepatitis	3.4	Icterus index 40. Prothrombin 45 per cent, died
4	Hepatitis	7.0	Postoperative
5	Alcoholic cirrhosis	6.9	
6	Alcoholic cirrhosis	4.0	Died 48 hours later
7	Cirrhosis, nonalcoholic	10.2	Proved by biopsy
8	Alcoholic cirrhosis	4.6	
	c. Mixed or Type Unknown		
1	Unknown	14.0	Intense jaundice
2	Lymphosarcoma	8.0	Extensive liver involvement
3	Cirrhosis (?)	9.2	
4	Diabetes	13.3	Slight jaundice
5	Obstruction (?)	8.2	
6	Unknown	12.5	
7	Carcinoma of esophagus	5.2	Jaundice postoperative

liver function, or the type of jaundice can be made in these few cases. Those with obstructive jaundice due to carcinoma of the pancreas had elevated values, as reported by Innerfield and associates[5] but apparently an increase can also occur in obstructive jaundice due to other causes. The marked deficiency of antithrombin found in some patients with hepatic jaundice is worthy of note. Patients with liver disease but without jaundice have not been examined, and no data are available at this time on patients with acute pancreatitis.

SUMMARY

A clinical method for determining antithrombin activity of plasma has been described.

With this technique, normal or elevated values for antithrombin were obtained in patients with thrombosis.

The majority of patients under treatment with bishydroxycoumarin had an increase in antithrombin activity.

A few patients with hemorrhagic diathesis had elevated antithrombin values.

Patients with jaundice showed a wide range of antithrombin activity.

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REFERENCES

1. Kay, J. H., Hutton, S. B., Weiss, G. M., and Ochsner, A.: Studies on an Antithrombin. III. Surgery **28:** 24-28, 1950.
2. Volkert, M., and Piper, J.: The Heparin Content of Blood in Clinical Thrombosis, Acta Chir. Scandinav. **89:** 417-425, 1943.
3. Hurn, M., Barker, M. W., and Mann, F.: Prothrombin and Antithrombin in Patients with Thrombosing Tendencies, Am. J. Clin. Path. **17:** 709-711, 1947.
4. Seegers, W. H.: Antithrombin—Alpha Tocopherol (Including discussion) Transactions of Fourth Conference on Blood Clotting and Allied Problems, pages 157-216, Josiah Macy, Jr., Foundation 1951.
5. Innerfield, I., Angrist, A., and Benjamin, J. W.: Plasma Antithrombin Patterns in Disturbances of the Pancreas, Gastroenterology **19:** 843-854, 1951.
6. Howell, W. H.: Purification of Heparin and Its Presence in Blood, Am. J. Physiol. **71:** 553-562, 1925.
7. Astrup, Tage: Biochemistry of Coagulation, Acta physiol. Scandinav. **7:** Supplement No. 21, 1944.
8. Klein, P. B., and Seegers, W. H.: The Nature of Plasma Antithrombin Activity, Blood **5:** 742-752, 1950.
9. Ware, A. G., and Seegers, W. H.: Two-Stage Procedure for the Quantitative Determination of Prothrombin Concentration, Am. J. Clin. Path. **19:** 471-481, 1949.
10. Stefanini, M.: Purification of Resin Amberlite IR 100 for Blood Coagulation Studies, Proc. Soc. Exper. Biol. & Med. **67:** 22-25, 1948.
11. Hurn, M., Barker, N. W., and Mann, F.: Prothrombin and Antithrombin Following Administration of Dicumarol, Am. J. Clin. Path. **17:** 712-718, 1947.

PATHOGENESIS OF THE PLASMA TRANSFUSION REACTION WITH ESPECIAL REFERENCE TO THE BLOOD COAGULATION SYSTEM*

LT. COL. WILLIAM H. CROSBY, MEDICAL CORPS, UNITED STATES ARMY,** AND MARIO STEFANINI, M.D.***
BOSTON, MASS.

DAMESHEK and Neber[11] have recently described a reaction that occurred in certain susceptible patients during the transfusion of compatible whole blood or plasma. Chill, fever, backache, and pain in the legs were common symptoms. There were no allergic signs, nor was there evidence of sudden intravascular hemolysis that accompanies incompatible transfusions. The factor that provoked the reaction was found to be in the plasma. Red cells washed free of plasma could be transfused with safety, but plasma from the same blood caused a sharp reaction when injected intravenously in a susceptible patient. When the plasma was heated to 56° C. for thirty minutes, its ability to induce the reaction was destroyed. The *plasma transfusion reaction* (PTR) has been encountered in patients with hemolytic disease such as acquired hemolytic anemia with abnormal antibodies, severe pernicious anemia, sickle-cell anemia, leukemia, and also in patients with aplastic anemia and terminal cancer. Most of these patients had received many transfusions before one was given that induced a PTR, but in some this reaction occurred during the first transfusion. In these diseases, the occurrence of PTR is an exceptional experience occurring rarely and unpredictably. In paroxysmal nocturnal hemoglobinuria, however, PTR is the rule. Unless the red cells are washed before transfusion a reaction follows almost invariably,[6, 8] an unfortunate circumstance because the disease is refractory to treatment, and many of these patients are so anemic that they require repeated transfusions to prevent their becoming invalids. So far as we have been able to learn, the PTR in nocturnal hemoglobinuria is the same sort of reaction that occurs in other forms of anemia mentioned above. The reaction, however, does not limit itself to the described subjective symptoms (chill, fever, backache), since, in nocturnal hemoglobinuria, a hemolytic crisis is an invariable sequel of PTR. Several hours after the chill hemoglobinuria appears, and it may last two or three days.

It has recently been shown that the plasma factor which destroys the abnormal red cells in nocturnal hemoglobinuria resembles in some respects

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**Hematology Department, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

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From the Ziskind Laboratories (Hematology Section) of the Joseph H. Pratt and the New England Center Hospitals and the Department of Medicine, Tufts College Medical School

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accelerator globulin, one of the factors of the coagulation system.[6] The hemolytic and accelerator factors are probably not identical, but in any case there exists a rapport between the hemolytic system in nocturnal hemoglobinuria and the mechanism of blood coagulation. This relationship has suggested that the PTR, which is hemolytic in nocturnal hemoglobinuria, might involve the coagulation system. It was therefore decided to investigate the behavior of various clotting factors during the PTR. Because of the number and complexity of the determinations to be made, it was impractical to depend upon fortuitous reactions. Patients with nocturnal hemoglobinuria react to transfusion with discouraging dependability. So, with the consent and cooperation of our patients, we deliberately induced the PTR and studied the changes that occurred throughout its duration.

MATERIALS AND METHODS

The histories have already been published of the three patients with nocturnal hemoglobinuria upon whom these investigations were carried out.[10, 11] The PTR was induced by giving transfusions of unwashed compatible red cells. Sometimes the severity of the reaction was mitigated by washing the red cells once or twice, a procedure insufficient to remove all trace of plasma protein. Several patients with other diseases, who developed unpremeditated PTR, were also studied in enough detail to be sure that the pattern of the reaction, excepting hemolysis, was similar to that observed in nocturnal hemoglobinuria.

Serial tests of most of the known factors involved in the coagulation system were carried out during and after the induced transfusion reactions. *Platelet counts* were performed by the indirect method of Dameshek,[9] which permits simultaneous enumeration of *reticulocytes. Red cell, white cell, and differential counts* were performed with the usual techniques in blood obtained from a finger puncture. *Capillary fragility* was determined by applying negative pressure of 180 mm. Hg for two minutes to an area 2 cm. in diameter on the volar surface of the forearm, two fingerbreadths below the antecubital fossa.[32] The petechiae that appeared within the circle were counted. *Coagulation time* was determined in glass test tubes and silicone-coated test tubes at 37° C. by a modification of Lee and White method.[25] *Clot retraction* was estimated after one hour of incubation at 37° C. on the basis of an arbitrary classification from 0 to 4 plus.

We investigated the activity of various factors involved in the second stage of coagulation (conversion of prothrombin to thrombin). *Prothrombin activity of plasma* diluted to 10 per cent with deprothrombinized plasma was determined by a modification of the one-stage method of Quick.[23] *The activity of plasma prothrombin conversion factor* (also called labile factor, plasma Ac globulin and factor V) was determined by a method previously published.[29] *Serum prothrombin activity* was studied by a one-stage method. *Serum accelerator activity* was determined by the method of de Vries and associates.[12] The deprothrombinized plasma used in these procedures was prepared by incubating fresh normal oxalated plasma with tricalcium phosphate gel.[30] The thromboplastin was a saline suspension of acetone-extracted human brain. It gave a prothrombin time of 10 seconds with undiluted plasma, and twenty-one to twenty-two seconds with plasma diluted 1:10 with deprothrombinized plasma. *Plasma fibrinogen concentration* was determined by a slight modification of Quick's method,[22] and *plasma fibrinolytic activity* by an original quantitative technique.[31]

Hemoglobin concentration in plasma, serum and urine was determined by Ham's modification[13] of the micromethod of Bing and Baker. The serum hemoglobin was measured after the clot had been incubated in a stoppered test tube for one hour at 37° C. The difference between plasma and serum hemoglobin was taken as an index of serum hemolytic activity. Urinary hemoglobin expressed as milligrams excreted per hour served as an index of hemolytic activity in vivo when the plasma hemoglobin concentration was above the renal threshold.

RESULTS

For convenience in describing the sequence of events in the PTR as it occurs in paroxysmal nocturnal hemoglobinuria, three phases are referred to: (1) chill, (2) fever, and (3) hemolytic sequel. The severity of the reaction varied in different patients. In the case of one woman who was extremely susceptible, several washings of the red cells with saline served only to lighten the attack. As many as six washings were needed to prevent it. There were also unexplained changes in the sensitivity of the patients. Sometimes unwashed red blood cells would induce a light reaction—sometimes a severe one. This sort of change occurred over long periods of time. The patients might be extremely sensitive for many months, then for several months the sensitivity would disappear. We were unable to correlate the change with any improvement of the patient's general condition. During febrile illness all of the patients seemed definitely to be more reactive.

The PTR always began with a vague sense of apprehension, often accompanied by a flash of coolness along the spine or a short-lived chill or vague headache. At times there was also a slight drop in body temperature and blood pressure. In a mild reaction, this was often the only clinical evidence. When the reaction was severe other signs and symptoms developed. The chil-

TABLE I. MODIFICATIONS OF VARIOUS BLOOD AND PLASMA FACTORS DURING THE COURSE OF PLASMA TRANSFUSION REACTION IN ONE PATIENT WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

	TIME* (HOURS)						
	0	1½	2¼	4	6	9	24
White cells, per cu.mm.	5,450	7,100	1,450	6,000	----	9,300	5,300
Neutrophils, per cent	53	40	55	88	----	87	70
Eosinophils, per cent	0	0	3	0	----	0	2
Basophils, per cent	0	2	0	0	----	0	0
Lymphocytes, per cent	35	45	43	11	----	11	16
Monocytes, per cent	12	13	0	1	----	2	12
Nucleated red blood cells (per 100 white blood cells)	1	2	9	1	----	1	1
Reticulocytes, per cent	4.1	5.7	7.2	5.2	----	3.0	0.9
Platelets, per cu.mm. (× 1,000)	160	40	89	154.5	----	194	172
Coagulation time (minutes)							
glass test tubes	9	8½	5	7	11	10	10
Silicone-coated tubes	25	24	14	21	32	27	22
Prothrombin activity, per cent							
plasma	100	90	95	72	85	100	80
serum	2	4	5	6	5	10	8
Activity of plasma prothrombin conversion factor (labile factor), per cent	100	100	135	90	100	100	95
Serum accelerator (per cent acceleration)	110	100	98	105	56	42	40
Plasma fibrinogen (mg. per 100 ml.)	432	403	321	407	462	434	----
Fibrinolytic activity of plasma (per cent)	4	27	81	94	12	2	----
Hemoglobin							
plasma (mg. per 100 ml.)	115	100	105	92	101	120	197
serum (mg. per 100 ml.)	248	268	384	213	474	632	375
urine (mg. per hour)	50	6	20	0	3	12	30

*The transfusion was begun at time 0 and completed in one hour. There was a shaking chill at two hours.

liness became a shaking chill and the temperature might rise as high as 103° F. The patients complained bitterly of abdominal pain, and borborygmi were audible. Nausea and vomiting occurred frequently. In the most severe reactions observed by us, there was diarrhea with guaiac-positive stools. Headache was the rule and it was often severe.* At the peak of a moderate or severe reaction cyanosis usually appeared and one patient always developed a dry troublesome cough. After the chill, and as the fever abated, the severe symptoms subsided, but for several hours the patients felt ill and shaken and complained of headache and abdominal pain. This pattern of PTR was uniform in all patients. The hemolytic phase of the reaction occurred only in nocturnal hemoglobinuria.

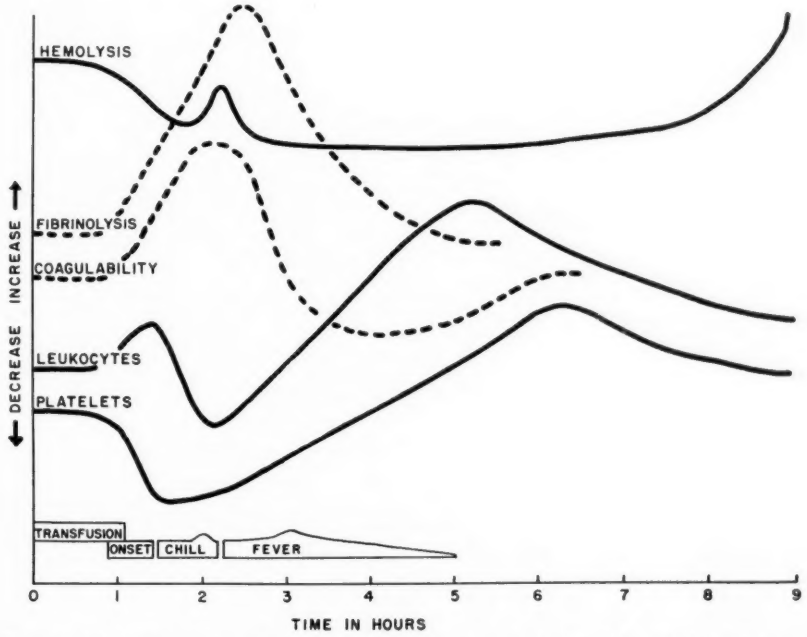

Fig. 1.—The curves represent the relation in time and intensity of the components of the plasma transfusion reaction. The ordinate is not calibrated, for the changes shown are only approximations based upon the experience with numerous reactions. For each curve the plateau at the left end is the base line for that curve and its distance from the abscissa is without significance.

During the phase of chill and fever, the hemolytic process of this disease was temporarily less active and hemoglobinuria, if present, disappeared (Fig. 1). After several hours and without subjective symptoms hemolytic activity would recommence, grow gradually more intense and might remain critically active for several days with hemoglobinuria day and night.

The laboratory findings presented in this paper were those observed in PTR of moderate severity. The results of a characteristic experiment are shown in Table I, and all of the results are summarized graphically in Fig. 1.

*Abdominal pain and headache should not be regarded lightly in nocturnal hemoglobinuria. Mesenteric thrombosis has been found at operation in patients complaining of severe abdominal pain. Severe headache has, at times, heralded the onset of hemiplegia. One of the patients studied in this investigation developed right hemiplegia in 1944 following a plasma reaction to transfused whole blood. Another patient of this series died recently with thromboses involving the splenic, portal, hepatic, and superior mesenteric veins.

The platelet count dropped at the time of the first vague chilly sensation. The reduction was rather pronounced, the platelet level falling from 150,000 to about 40,000 per cubic millimeter. The initial figure is low because patients with nocturnal hemoglobinuria ordinarily have a low platelet count. On dry smears taken at the time of the rapid drop the platelets showed a definite tendency to form rather tight little clumps. The clumps were not noted in wet preparations, probably because the blood was diluted with a solution containing sodium citrate. The platelet count recovered rapidly after the subsidence of chill and fever. The presence of giant platelets, 5 to 8 μ in diameter, was observed at this time. No changes in capillary fragility were demonstrated except on one occasion when the patient initially had a marked thrombocytopenia (50,000 platelets per cubic millimeter). The test done before transfusion produced 15 petechiae, evidence of slightly increased capillary fragility. After the onset of the febrile reaction the test was repeated and only four petechiae appeared. It is interesting that capillary resistance increased in spite of a further sharp decline in the number of circulating platelets.

Coagulation time was consistently shortened during the chill. As the patient recovered, the coagulation time returned to its original level and then became slightly prolonged so that clotting times up to eighteen minutes in glass test tubes were observed shortly after the rigor had subsided. In cases of particularly severe hemolytic reaction, the coagulation time was found to be still prolonged after twenty-four hours. The changes of clotting time in silicone-coated tubes were exaggerations of the pattern observed in ordinary glass test tubes.

Plasma prothrombin activity was moderately decreased during the febrile reaction. Values dropped from 100 per cent of normal to 90, 70, or 50 per cent, but quickly returned to the original level after the febrile phase had passed. During severe hemolytic sequelae the prothrombin time was sometimes prolonged. When oxalated plasma taken during this phase of the reaction was incubated at 4° C. for several hours, the previously delayed prothrombin time was found to have become normal. Plasma prothrombin activity was completely unchanged during mild PTR.

Serum prothrombin activity is regarded as an indirect index of prothrombin consumption during coagulation.[30] After the chill there was a constant gradual increase of serum prothrombin activity which suggested that a slight impairment of prothrombin consumption was developing during recovery from the febrile phase of the PTR.

The activity of prothrombin conversion factor (plasma Ac globulin, factor V, labile factor, proaccelerin, etc.) often increased slightly from 100 to 110-115 per cent of normal activity during the height of the febrile reaction. On the occasion described in Table I, it went as high as 135 per cent. The increase in activity coincided with the shortening of the clotting time and it always subsided rapidly.

Serum accelerator activity did not vary to any great extent during the febrile reaction, but declined markedly as the hemolytic phase set in. It is

interesting that sera collected during the hemolytic reaction, if incubated at 4° C., showed a gradual increase of accelerator activity. In the experiment presented in Table I, the serum collected four and one-half hours after the completion of the transfusion had an accelerator activity of 56 per cent at that time. After an hour at 4° C. the accelerator activity was found to be 84 per cent, and it was 150 per cent after eighteen hours. The prothrombin activity of the same serum was 4.5 per cent immediately after separation from the clot. It was unchanged after one hour, and was 6.5 per cent after eighteen hours.

Plasma fibrinogen concentration decreased during the febrile reaction. It was suspected that a large part of the apparent loss was due to increased fibrinolytic activity of the plasma and occurred after calcium had been added to the plasma to convert all fibrinogen to fibrin. Therefore, the *rate* of fibrinolysis in vitro was established and extrapolation of values showed that the loss of fibrin was not all artefactual. Somewhat less than half of the recorded decrease in fibrinogen probably occurred in vivo, the rest while the test was being performed. Our earlier statement[7] that plasma fibrinogen was halved during the PTR was excessive since our interpretation did not allow for the fibrinolytic activity of serum in vitro. As much as half of the total fibrinogen was destroyed in vivo during the PTR. A patient whose plasma volume is approximately 3,000 ml. and whose initial fibrinogen concentration is 400 milligrams per 100 ml. has a total fibrinogen of 12 grams. During a moderate PTR the loss of this protein from the circulation may amount to about 5 grams.

Plasma fibrinolytic activity usually appeared at the time of the chill and rapidly increased. It persisted during the febrile phase and faded at the onset of the hemolytic reaction. At the height of this activity, a quantitative technique demonstrated that over 90 per cent of the fibrin was lysed within two hours. Blood clots that had formed spontaneously in vitro collapsed after thirty to sixty minutes of incubation at 37° C.

Clot retraction was usually poor during the febrile phase. This corresponded with the fall in platelet count and also with development of plasma fibrinolytic activity. During the fibrinolytic reaction clots would often disappear before retraction had become complete.

Plasma hemoglobin concentration, always abnormally high in nocturnal hemoglobinuria, presents a characteristic nocturnal-diurnal rhythm in that disease. The plasma hemoglobin level rises during sleep sometimes high enough to cause "nocturnal" hemoglobinuria and falls rather rapidly after the patient awakes. In this disease, therefore, the daily fluctuations of the plasma hemoglobin must be taken into account. Prior to transfusion, serial studies of the plasma hemoglobin level in our patients were conducted for several days and nights. Comparison of these results with those obtained on the day of the transfusion showed that the changes associated with the PTR were not related to the nocturnal-diurnal rhythm. At the onset of the PTR febrile phase, plasma hemoglobin level fell and hemolytic activity remained low for several hours. During severe reactions the plasma hemoglobin level would rise for a short time after the initial drop, usually during the shaking chill. In all cases a marked elevation of the plasma hemoglobin occurred later, at the onset of the hemolytic

phase (Fig. 1). No variations in the plasma hemoglobin concentration were noted when our patients with nocturnal hemoglobinuria were transfused with saline-washed red cells, thereby avoiding the PTR.

The *serum hemoglobin concentration* usually fell during the febrile reaction and rose, often steeply, after the hemolytic phase began. During the temporary elevation of plasma hemoglobin, often noted with the shaking chill, the serum hemoglobin showed a similar but more pronounced peak.

The *urinary hemoglobin*, in milligrams per hour, followed closely the variations of plasma and serum hemoglobin. We considered this to be the most accurate indicator of the variations of hemolysis in vivo, but, of course, it was available only when the hemolytic reaction was severe enough to bring the plasma hemoglobin concentration above the renal threshold. In general, the hemoglobin determinations in plasma, serum, and urine paralleled one another in a most satisfactory manner.

The *white cell count* rose slightly at the onset of the PTR only to fall precipitately during the chill. All types of white cells were involved in this fall, but the monocytes seemed to be especially susceptible. During recovery from the febrile phase leukocytosis developed, almost entirely due to reappearance of neutrophils. The number of monocytes and lymphocytes remained low for at least 24 hours after the onset of the reaction. At the beginning of the febrile episode, numerous smudge cells were observed on dry smears.

Nucleated Red Cells and Reticulocytes.—The patient whose reaction is illustrated in the table had undergone splenectomy ten years previously. One or two nucleated red cells could usually be found in his peripheral blood. The febrile reaction in this patient was accompanied by elevation of the number of reticulocytes and nucleated red cells and the appearance of basophilic erythroblasts. The reticulocytes and nucleated red cells disappeared as the fever dropped and the hemolytic phase began.

DISCUSSION

1. *Changes in Coagulability of the Blood During PTR.*—At first glance it seems paradoxical that the coagulation time of the blood should be shortened during the chill for, at this time, the platelets, prothrombin, and fibrinogen all had markedly decreased. Further evidence of hypercoagulability is found in the results of the test for the plasma prothrombin conversion factor (labile factor). During the chill, the results of this test indicated an apparent increase of activity from the normal 100 per cent to as much as 135 per cent. It cannot be concluded from such results that labile factor is actually increased. Although a deficiency of this factor prolongs the clotting time of plasma in the test, it does not follow that an excess will cause coagulation in a shorter time. Indeed, if an excess of the factor is artificially added to the test in vitro the clotting time is not shortened beyond that point which represents 100 per cent of activity. In the present instance, the apparent increase of labile factor activity must be due to the intrusion of some other agent which is able to hasten clotting, perhaps the same agent that shortens the coagulation time of whole blood.

The fall in the platelet count and the loss of prothrombin activity and fibrinogen from circulating blood could also be regarded as signs of increased coagulability. There would seem to occur *an attempt at intravascular coagulation.* One might postulate that the PTR induces platelet injury. The platelets, by agglutinating and disintegrating, form thromboplastin. If this reaction were of sufficient amplitude and velocity it would overcome the safeguards of the coagulation system and thrombin would be formed and, finally, fibrin. The results of such activity would appear in our battery tests as a drop in platelet count followed by decreased prothrombin activity and diminished fibrinogen concentration (prothrombin and fibrinogen being consumed during the coagulation of blood) all associated with increased blood coagulability. This actually occurred in our patients. These suggestions find support in the work of Landois.[16] In dogs that died as a consequence of transfusions of heterologous blood, he found that blood vessels were blocked with fibrin emboli and ante mortem clots. If the PTR involves a similar attempt at intravascular coagulation, it is a comparatively mild one and reversible. Fibrinolysin appears to destroy the fibrin that has been formed, and soon afterward, the coagulation time becomes prolonged beyond the normal. This hypocoagulability suggests the appearance of an anticoagulant. In other types of hemoclastic reaction heparin has been shown to be present.[14, 21]

The possibility that a general injury of the circulating platelets may act as a trigger mechanism for an attempt at intravascular coagulation is not fantastic since Tocantins[34] produced severe hemoclastic reactions in rabbits by injection of antiplatelet sera. It may be that the platelets in nocturnal hemoglobinuria are exceptionally sensitive[4] and, as a consequence, patients are prone to develop PTR.

2. *The Fibrinolytic Reaction.*—Fibrinolysin exists in plasma as an inert proenzyme. Fibrinolytic activity occasionally appears in vivo after injection of adrenalin or during emotional stress,[17] and it has been postulated that activation of fibrinolysin is one facet of the "alarm reaction" of Selye. The appearance of fibrinolytic activity is an outstanding phenomenon not only of the PTR but of all "hemoclastic reactions" and, indeed, of many sorts of severe tissue injury, since many tissues contain the activator of fibrinolysin, fibrinokinase. The lung appears to be an especially fertile source of this activator.[33] Soulier and associates[27] have reported a series of cases in which a devastating increase in fibrinolytic activity occurred during pulmonary surgery and caused uncontrollable hemorrhage. There is no evidence to suggest that the lung is the source of fibrinolytic activity in PTR, but it may be. The leukocytes that disappear from the blood during hemoclastic reactions are presumably taken up by the lung. Andrewes[1] induced an acute leukopenia by injecting tuberculin into normal rabbits and found that pulmonary capillaries were filled with white cells. Recently, Weisberger and associates,[36] using leukocytes tagged with radiophosphorus, have shown that the lung rapidly removes most of the transfused white cells from the blood together with a fair proportion of native white cells. During PTR the lung is probably removing injured leukocytes and it may also be bombarded by platelet emboli and shreds of fibrin, as many of the platelets

disappear from the circulation and perhaps 5 Gm. of fibrinogen are "consumed" intravascularly during a moderate PTR. Injury of the lung may explain the appearance in the blood of a powerful activator of fibrinolysin. Blocking of pulmonary capillaries may also explain the cyanosis and the troublesome dry cough that developed in our patients.

3. *The Involvement of Other Organs in PTR.—Bone Marrow:* The shower of reticulocytes and nucleated red cells into the peripheral blood could be regarded as evidence of bone marrow involvement. It is interesting to note that the immature red cells appear at the time of the chill when platelets and white cells are being swept from circulation and before the replacement of these other elements has begun. The reticulocytes and nucleated red cells of the paroxysm disappear rapidly. They are gone within several hours—not because they become mature in that short time, but probably because they are exceptionally fragile.

The reason for the bone marrow involvement is not clear. Perhaps it is related to the cyanosis that develops. Alternatively, the reaction may be due to the lodging of emboli composed of fibrin or platelets in the capillaries of the marrow.

The portal system: In Landois'[16] dogs that died after transfusion with heterologous blood, hyalin emboli and ante mortem clots were found in the intestinal mesentery. These dogs, before they died, developed hyperperistalsis and bloody diarrhea. In PTR the same mechanism—reversible in this case—may have caused the abdominal pain, borborygmi and diarrhea that developed in our patients.

The central nervous system: The headaches, chills, and fever may also have been due to tiny emboli which obstructed the capillaries of various parts of the central nervous system.

All of these signs and symptoms indicate that a widespread injury has occurred involving many of the organs of the body.

4. *The Hemolytic Crisis.*—Only in nocturnal hemoglobinuria have we observed a hemolytic crisis as a sequel to the PTR. The hemolytic process involves the erythrocytes of the patient and not those of the donor. The erythrocyte in nocturnal hemoglobinuria is abnormal in that it is susceptible to hemolysis by a normal component of human plasma. The plasma factor may be activated in vitro by thrombin, thromboplastin, platelet materials, and dilute heparin.[6] In vivo, its activity is usually slight but enough to cause a chronic hemolysis of the defective red cells of nocturnal hemoglobinuria. Intense activity develops during the hemolytic crises that are precipitated by infection, physical injury, or the PTR.

During the phase of chill and fever in PTR, the chronic hemolytic activity of nocturnal hemoglobinuria is temporarily inhibited (Fig. 1). If hemoglobinuria were present when the reaction began it now stops. The plasma hemoglobin falls and the serum hemolytic activity is diminished. The reason for this is not known, but it may be significant that the period of inhibition always coincides with the period of increased fibrinolytic activity. Injection of adrenalin which activates fibrinolysin is also able to inhibit, for a short time, the hemolysis

in nocturnal hemoglobinuria.[35] The parallel appears to be significant although one cannot conclude that fibrinolysin blocks this hemolytic reaction. During a severe PTR, the period of inhibited hemolysis is interrupted by a short, sharp hemolytic reaction at the time of the shaking chill (Fig. 1). The pathogenesis of this flurry of activity was not studied.

As the fever subsides the reaction in patients with nocturnal hemoglobinuria enters a hemolytic phase. Plasma hemoglobin rises, hemoglobin appears in the urine and the hemolytic activity of the serum becomes exceptionally destructive. At the same time, tests for the coagulation factors demonstrate a minor degree of inhibition. Prothrombin consumption is slightly, but consistently, inhibited and serum accelerator activity is definitely reduced. Yet, if the serum is incubated at 4° C., accelerator activity gradually appears as though it had been masked. The presence of a heparinlike substance in the blood during this phase of the reaction is suspected. Although it has not been demonstrated in the PTR, heparin is known to appear in other types of hemoclastic reaction.[14]

5. *The Significance of Plasma Transfusion Reaction.*—Dameshek and Neber[11] postulated that the PTR was due to the activity of a heat-labile factor present in normal human plasma. They found no immune antibody to account for the reaction, a circumstance that had been noted earlier by Dacie.[8] The unknown plasma factor acts on the blood of the susceptible patient to produce a series of changes which are characteristic of the PTR. While most of these involve the coagulation mechanism, there are others which are also noteworthy. Leukopenia develops, there is a reticulocyte shower, and, in nocturnal hemoglobinuria, the hemolytic system is profoundingly modified. Clinical signs include fever, cyanosis, and hyperperistalsis. A similar pattern of reactions may be touched off by a number of noxious stimuli, either in normal human beings and animals, or in especially sensitized subjects. Thus, an injection of peptone or typhoid bacilli or the transfusion of incompatible blood can produce this reaction pattern in any human being or animal. The anaphylactic shock that occurs when an antigen is injected into an immunized animal is accompanied by the same changes that we have described for PTR. The injection of saccharated iron oxide, innocuous for most people, may produce this reaction in certain sensitive patients.[5] The paroxysms of cold hemoglobinuria are accompanied by changes indistinguishable from those of PTR. In the past, many of the characteristic features of PTR have been described in association with a variety of reactions. Silbermann[26] noted leukopenia during reactions to transfusions of incompatible blood and also during the paroxysms of cold hemoglobinuria. Pratt[20] described thrombocytopenia during peptone shock. Civaleri[3] found marked leukopenia in rabbits during anaphylactic shock and observed a shower of reticulocytes and nucleated red cells during the paroxysm of the reaction. More recently, Young and associates[38] have reported hypoprothrombinemia and leukopenia in dogs transfused with incompatible blood or plasma. Hypoprothrombinemia had been previously noted in the course of anaphylactic reactions,[39] and it has also been observed in peptone shock.[19] A most complete investigation of this type of reaction was conducted by Widal, Abrami, and Brissaud[37] on patients with paroxysmal cold hemoglobinuria. The reaction they described was almost identical to

that reported in this communication. During the patient's chill, the clotting time grew short, recovered, and then became prolonged. The plasma developed fibrinolytic activity. Clot retraction was impaired, and there was a sharp fall in the leukocyte count. Widal and co-workers recognized that the reaction was similar to that observed in anaphylactic and peptone shock. Because it involved profound disturbances in the plasma protein they suggested that it be called a *colloidoclastic reaction* or, because the formed elements of the blood were also involved, a *hemoclastic reaction*. Many other findings have been reported in the course of a hemoclastic reaction. Thus a drop of hemolytic complement activity occurs after injection of antigens in sensitized animals,[18, 28] and after paroxysms of cold hemoglobinuria in man.[15] The hemoclastic reaction due to peptone or reinjection of antigen is accompanied by release of heparin and histamine.[2, 14] Antifibrinolysin is reduced during peptone shock.[19]

It is difficult to escape the conclusion that the pattern of the PTR is entirely nonspecific, and an expression of the effect of many entirely unrelated stimuli. Even in paroxysmal nocturnal hemoglobinuria, patients have developed hemoclastic reactions not only from plasma but from injections of pooled serum, peptone, milk, Evans Blue, nitrogen mustard, sodium lactate, arsphenamine, liver extract, and many other chemical and biological materials.

SUMMARY AND CONCLUSIONS

The plasma transfusion reaction (PTR), described by Dameshek and Neber,[11] occurs when susceptible patients are transfused with compatible blood. They develop chills, fever, cyanosis, intestinal hyperperistalsis, and pains in various parts. This reaction is encountered in hemolytic diseases, terminal cancer and in paroxysmal nocturnal hemoglobinuria when the reaction is always followed by a hemolytic crisis. The PTR is evoked by a heat-labile plasma factor. Injection of plasma alone can induce it, but red cells washed free of plasma may be transfused with impunity. The plasma factor has not been identified.

PTR was induced in three patients with nocturnal hemoglobinuria. Various factors were studied. At the time of the chill, the blood became hypercoagulable although platelet count, prothrombin activity, and fibrinogen concentration were, at the same time, much reduced, suggesting that a generalized attempt at intravascular coagulation occurred during this phase. Thereafter, intense fibrinolytic activity rapidly developed in the plasma. An unidentified anticoagulant activity appeared.

The symptoms of the PTR may be due to embolism. During the preliminary phase of the reaction, the majority of the leukocytes and platelets are swept out of the circulation and about half of the fibrinogen disappears. The white cells are probably removed by the lungs. Emboli composed of platelets or fibrin may be responsible for the various clinical symptoms such as diarrhea, headache, and abdominal pain. Small emboli, by injuring the bone marrow, may also be responsible for the shower of reticulocytes and nucleated red cells which is associated with the PTR. The same reaction in the lung may explain the appearance of intense fibrinolytic activity in the plasma.

PTR is nonspecific. The same pattern of changes which we have described has been found during anaphylactic and peptone shock, incompatible transfusion, and the intravenous administration of foreign proteins such as tuberculin and typhoid bacilli. It occurs following the injection of iron compounds in susceptible people and during the hemolytic paroxysms of cold hemoglobinuria, favism and blackwater fever. This pattern has been described as a colloidoclastic or hemoclastic reaction.

REFERENCES

1. Andrewes, F. H.: The Behavior of the Leukocytes in Infection and Immunity, Lancet **2**: 8, 1910.
2. Carryer, H. M., and Code, C. F.: Release of Histamine During Hemolytic Reactions in the Blood of Rabbits, Proc. Soc. Exper. Biol. & Med. **73**: 452, 1950.
3. Civaleri, I.: Le Emoglobinurie, Siena, 1916.
4. Crosby, W. H.: Pathogenesis of Leukopenia and Thrombocytopenia in Paroxysmal Nocturnal Hemoglobinuria, Communication N. 95, Comptes Rendus du Troisième Congrès de la Société Internationale Européenne d'Hématologie, E. M. E. S., Rome, 1952.
5. Crosby, W. H.: Personal observation.
6. Crosby, W. H., and Dameshek, W.: Paroxysmal Nocturnal Hemoglobinuria. The Mechanism of Hemolysis and Its Relation to the Coagulation Mechanism, Blood **5**: 822, 1950.
7. Crosby, W. H., and Stefanini, M.: Pathogenesis of Plasma Transfusion Reaction, Fed. Proc. **9**: 26, 1950.
8. Dacie, J. V.: Transfusion of Saline-washed Red Cells in Nocturnal Hemoglobinuria. (Marchiafava-Micheli disease), Clin. Sc. **7**: 65, 1948.
9. Dameshek, W.: Method for Simultaneous Enumeration of Blood Platelets and Reticulocytes, Arch. Int. Med. **50**: 579, 1932.
10. Dameshek, W.: Paroxysmal Nocturnal Hemoglobinuria, Bull. New England M. Cent. **4**: 224, 1942.
11. Dameshek, W., and Neber, J.: Transfusion Reactions to a Plasma Constituent of Whole Blood. Their Pathogenesis and Treatment by Washed Red Cell Transfusions, Blood **5**: 129, 1950.
12. de Vries, A., Alexander, B., and Goldstein, R.: A Factor in Serum Which Accelerates the Conversion of Prothrombin to Thrombin: I. Its Determination and Some Physiologic and Biochemical Properties, Blood **4**: 247, 1949.
13. Ham, T. H.: A Syllabus of Laboratory Examinations in Clinical Diagnosis, Harvard University Press, 1950.
14. Jaques, L. B., and Waters, E. T.: The Identity and Origin of the Anticoagulant of Anaphylactic Shock in the Dog, J. Physiol. **99**: 454, 1941.
15. Kumagi, T., and Inoue, B.: Beitrage zur Kenntnis der paroxysmalen Hämoglobinurie, Deutsche med. Wchnschr. **38**: 361, 1912.
16. Landois: Die Transfusion des Blutes, Leipzig, 1875.
17. MacFarlane, R. G., and Biggs, R.: Fibrinolysis. Its Mechanism and Significance, Blood **3**: 1167, 1948.
18. M'Gowan, J. P.: In Vivo Experiments with Complement, J. Path. Bact. **12**: 519, 1908.
19. Nolf, P., and Adant, M.: Le choc anaphylactique du chien hépatectomisé, Communication N. 141, Comptes Rendus du Troisième Congrès de la Société Internationale Européenne d'Hématologie, E. M. E. S., Rome, 1952.
20. Pratt, J. H.: Beobachtungen über die Geringungszeit des Blutes und die Blutplattchen, Arch. Exper. Path. Pharmakol. **49**: 299, 1903.
21. Quick, A. J.: On the Coagulation Defect in Peptone Shock, Am. J. Physiol. **116**: 535, 1936.
22. Quick, A. J.: The Physiology and Pathology of Hemostasis, Philadelphia, 1951, Lea & Febiger, p. 156.
23. Quick, A. J.: On the Quantitative Estimation of Prothrombin, Am. J. Clin. Path. **15**: 560, 1945.
24. Quick, A. J., and Stefanini, M.: The State of Component A (Prothrombin) in Human Blood; Evidence That It Is Partly Free and Partly in an Inactive or Precursor Form, J. Lab. & Clin. Med. **34**: 1203, 1949.
25. Quick, A. J., Honorato, C. R., and Stefanini, M.: The Value and the Limitations of the Coagulation Time in the Study of the Hemorrhagic Diseases, Blood **3**: 1120, 1948.
26. Silbermann, O.: Zur Pathogenese der essentiellen Anämien. Ein experimenteller und klinischer Beitrag, Berlin. klin. Wchnschr. **23**: 473 and 492, 1886.

27. Soulier, J. P., Mathey, J., Le Bolloch, A. G., Daumet, Ph., and Faye, H.: Syndromes Hémorragiques Mortels avec Incoagulabilité Totale par Défibrination et avec Fibrinolyse. I. Au Cours des Exéreses Pulmonaires, Rev. hémat. **7**: 30, 1952.
28. Stavitsky, A. B., Stavitsky, R., and Ecker, E. E.: Loss of Hemolytic Complement Activity and of Granulocytes Following Reinjection of an Antigen into a Rabbit, J. Immunol. **63**: 389, 1949.
29. Stefanini, M.: New One-stage Procedures for the Quantitative Determination of Prothrombin and Labile Factor, Am. J. Clin. Path. **20**: 233, 1950.
30. Stefanini, M., and Crosby, W. H.: The One-stage Prothrombin Consumption Test. Clinical Value in the Identification of Thromboplastin-deficiency Diseases, Blood **5**: 962, 1950.
31. Stefanini, M., Crosby, W. H., and Salomon, L.: In preparation.
32. Stefanini, M., and Petrillo, E.: The Relative Importance of Plasmatic and Vascular Factors of Hemostasis in the Pathogenesis of the Hemorrhagic Diathesis of Liver Dysfunction, Acta med. Scandinav. **134**: 139, 1949.
33. Tagnon, H. J., and Palade, G. E.: Activation of Proplasmin by a Factor from Mammalian Tissue, J. Clin. Investigation **29**: 317, 1950.
34. Tocantins, L. M.: Experimental Thrombocytopenic Purpura; Cytological and Physical Changes in the Blood, Ann. Int. Med. **2**: 838, 1936.
35. Villa, L.: Il significato semeiologico della contrazione di volume della milza mediante adrenalina, Cuore e Circ. **12**: 465, 1928.
36. Weisberger, A. S., Heinle, R. W., Storaasle, J. P., and Hannah, R.: Transfusion of Leukocytes Labeled with Radioactive Phosphorus, J. Clin. Investigation **29**: 336, 1950.
37. Widal, F., Abrami, P., and Brissaud, E.: L'autoanaphylaxie. Son rôle dans l'hémoglobinurie paroxystique. Traitement antiphylactique de l'hémoglobinurie. Conception physique de l'anaphylaxie. Sem. med. **33**: 613, 1913.
38. Young, L. E., Ervin, D. M., and Yuile, C. L.: Hemolytic Reactions Produced in Dogs by Transfusion of Incompatible Dog Blood and Plasma, Blood **4**: 1218, 1949.
39. Zunz, E., and La Berre, J.: Contribution à l'étude des modifications de la coagulation du sang au cours du choc anaphylactique chez le chien, Arch. intern. physiol. **25**: 221, 1925.

THE EFFECT OF NITROGEN MUSTARD (BIS-β-CHLOROETHYL AMINE) ON LIVER FUNCTION AND STRUCTURE IN PATIENTS WITH NEOPLASTIC DISEASE*

HYMAN J. ZIMMERMAN, M.D., OMAHA, NEB., LOUIS K. ALPERT, M.D., AND JOHN S. HOWE, M.D., WASHINGTON, D. C.

THE administration of a cytotoxic material such as nitrogen mustard might be expected to result in liver damage. This is an especially important consideration, since this agent is used in patients who may be severely ill and who frequently show impairment of liver function. Indeed, Damashek, Weisfuse, and Stein[4] have recommended that nitrogen mustard not be administered to patients who have "jaundice indicating some degree of hepatic dysfunction."

In order to evaluate the effect of nitrogen mustard on the liver, studies were made of liver function and structure before and after the administration of this agent to a group of patients with neoplastic diseases.

METHODS AND PROCEDURE

Fifteen patients with miscellaneous neoplastic diseases were included in this study (Table 1). All received nitrogen mustard according to the conventional plan of four daily administrations of 0.1 mg. per kilogram of body weight intravenously.

In all fifteen patients tests of liver function were performed one or two days before the administration of the nitrogen mustard and three to six days after completion of therapy. The procedures used included the bromsulfalein excretion (5 mg. of dye per kilogram of body weight, with the estimation of retention at forty-five minutes), the thymol turbidity test, and the cephalin-cholesterol flocculation. In four patients specimens of the liver for histologic examination were obtained by the needle biopsy technique. In G. A., W. W., H. C., and L. P. the specimens were obtained on the day before the beginning, and three days after the completion of therapy.

RESULTS

Ten of the fifteen patients initially showed an abnormal degree of bromsulfalein retention, and in all of these the degree of abnormality became less after treatment with nitrogen mustard. Two of these patients were jaundiced before the administration of nitrogen mustard. W. W., a patient with reticulum-cell sarcoma, whose biopsy specimen showed sarcomatous infiltration of the liver, had 5.5 mg. of bilirubin per 100 ml. of serum before treatment and 2.7 mg. per 100 ml. after treatment. Similarly, L. P., who had lymphocytic leukemia with leukemic infiltration of the liver, exhibited a decrease in serum bilirubin from 3.7 mg. per 100 ml. before treatment to 1.5 mg. per 100 ml. after therapy with nitrogen mustard.

From the Veterans Administration Hospital, Omaha, Neb., and the Veterans Administration Hospital, Washington, D. C.

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TABLE I. STUDIES OF LIVER FUNCTION IN PATIENTS WITH NEOPLASTIC DISEASE BEFORE AND AFTER NITROGEN MUSTARD THERAPY

		LIVER FUNCTION STUDIES							
		BEFORE HN_2 THERAPY				AFTER HN_2 THERAPY			
PATIENT	DIAGNOSIS	BSP. (PER CENT)	T.T.	C.F.	TOTAL SERUM BILIRUBIN	BSP. (PER CENT)	T.T.	C.F.	BILIRUBIN
H. M.	Hodgkin's disease	4	0.4	0	0.5	0	0.5	0	0.5
B. L.	Hodgkin's disease	12	–	0	0.5	8	–	0	–
H. G.	Hodgkin's disease	4	2.1	0	–	3	1.6	0	–
E. B.	Hodgkin's disease	10	4.1	4+	1.3	6	4.1	4+	0.35
R. W.	Hodgkin's disease	5	–	1+	–	0	–	1+	–
D. P.	Hodgkin's disease	15	0.5	0	0.6	6	0.4	0	0.3
M. M.	Granulocytic leukemia	4	2.1	1+	–	8	1.8	2+	–
H. C.	Lymphocytic leukemia	10	1.4	3+	0.5	8	1.0	3+	0.6
L. P.	Lymphosarcoma	38	2.0	1+	3.7	26	2.1	0	1.5
R. B.	Giant follicular lymphoma	12	2.0	1+	–	5	2.0	1+	–
W. W.	Reticulum-cell sarcoma	42	2.2	0	5.5	25	1.7	2+	2.7
G. A.	Bronchogenic carcinoma	0	5.0	1+	–	0	4.0	1+	–
F. P.	Bronchogenic carcinoma	2	1.6	3+	–	2	2.0	3+	–
C. P.	Bronchogenic carcinoma	15	0.4	0	0.7	11	0.5	1+	0.3
H. M.	Bronchogenic carcinoma	12	4.0	1+	–	6	5.0	3+	–

Of the remaining five patients who initially had normal bromsulfalein excretions, all but one showed no significant change. This patient (M. M.) showed an increase of dye retention from 4 to 8 per cent.

The flocculation tests in this group were normal, or only slightly abnormal, before treatment, and no significant changes were noted after treatment.

Of the four patients in whom histologic studies were performed, the three with lymphomas were found to have lymphomatous infiltration of the liver, while the one with carcinoma showed no malignant involvement of the liver in the biopsy specimen. In none of the patients in this group was there any significant difference between the pre- and post-treatment specimens. There was neither evidence of damage to the liver cells, nor decrease in the degree of lymphomatous infiltration.

DISCUSSION

In a wide personal experience with the use of nitrogen mustard,[1-3] no clinical evidence of liver damage has been noted. Damashek and his associates,[4] however, have reported aggravation of liver dysfunction in several patients, as well as the finding of miliary foci of necrosis in the livers of three patients dying between nine and nineteen days after the completion of therapy. They concluded that "the only definite contraindication to the use of HN_2 is the presence of jaundice indicating some degree of hepatic dysfunction."

Our observations are at variance with the data recorded by Damashek and associates. In the patients who showed impairment of liver function initially, there was a consistent decrease in the degree of abnormality. No

significant deterioration of liver function occurred in any of the patients studied. Furthermore, in the two patients with clinical jaundice, the degree of icterus decreased and the liver function tests improved after treatment. In the sections of liver tissue obtained from four patients before and after treatment with nitrogen mustard, no change in the liver parenchyma could be seen. Graef and his associates[5] reported only minimal fatty metamorphosis of the liver in animals receiving nitrogen mustard.

The present study, therefore, would seem to corroborate the clinical impression of lack of toxicity of nitrogen mustard for the liver, and would suggest that, when hepatic function is impaired in lymphomas due to infiltration of the liver, improvement may be anticipated following the administration of nitrogen mustard.

SUMMARY AND CONCLUSIONS

The effect of nitrogen mustard on liver function and structure was evaluated in a group of patients with neoplastic diseases. Of fifteen patients studied, ten showed improvement in liver function, five no change, and one mild aggravation. No significant histologic changes following the administration of nitrogen mustard were noted in the patients studied by needle biopsy. It is concluded that the impairment of liver function frequently seen in lymphomas is not a contraindication to the use of nitrogen mustard.

REFERENCES

1. Alpert, L. K., Greenspan, E. M., and Peterson, S. S.: Treatment of Lymphomas and Other Neoplastic Diseases With Nitrogen Mustard, Ann. Int. Med. **32**: 393-432, 1950.
2. Alpert, L. K., and Peterson, S. S.: Use of Nitrogen Mustard in Treatment of Lymphomata, Bull. U. S. Army Med. Dept. **7**: 187-194, 1947.
3. Alpert, L. K., Zimmerman, H. J., and Scherr, E. H.: The Effects of ACTH, Cortisone and Nitrogen Mustard on Malignant Diseases: Proceedings Second Annual ACTH Conference, John R. Mote, Editor, Philadelphia, 1951, The Blakiston Company, pp. 235-250.
4. Damashek, W., Weisfuse, L., and Stein, T.: Nitrogen Mustard Therapy in Hodgkin's Disease: An Analysis of Fifty Consecutive Cases, Blood **4**: 338-379, 1949.
5. Graef, O., Karnofsky, D. A., Valjager, B., Krichesky, B., and Smith, H. W.: Clinical and Pathologic Effects of the Nitrogen and Sulphur Mustard in Laboratory Animals, Am. J. Path. **24**: 1-38, 1948.

CARDIOPULMONARY STUDIES IN PATIENTS WITH MITRAL STENOSIS

I. Cardiovascular Dynamics

G. Miller, M.D., H. Goldberg, M.D., E. I. Elisberg, M.D., G. L. Snider, M.D., M. Toor, M.D., and L. N. Katz, M.D.
Chicago, Ill.

ALTHOUGH a number of earlier attempts at surgical correction of mitral valve deformities were made,[1-4] it is only recently that mitral valve surgery has become sufficiently successful to be widely adopted.[5-8] A knowledge of the cardiovascular and pulmonary alterations associated with mitral stenosis is of considerable importance in understanding the disease and in aiding in the selection of suitable candidates for surgery.

Several reports have appeared[9-19] dealing with cardiovascular findings in mitral stenosis. In view of the importance of these observations, additional studies on the cardiopulmonary function of patients with mitral stenosis were considered desirable. The present report deals with the cardiovascular dynamics of patients with mitral stenosis at rest and during exercise.

MATERIAL AND METHODS

Twenty patients who had rheumatic heart disease with mitral stenosis as the predominant valvular lesion were studied. The diagnosis of mitral stenosis was based on the criteria of the New York Heart Association.[20] All patients had an apical diastolic murmur. This was the only murmur heard in eight patients (Nos. 2, 5, 7, 8, 12, 13, 15, and 17), while twelve patients had an apical systolic murmur in addition (Nos. 1, 3, 4, 6, 9, 10, 11, 14, 16, 18, 19, and 20). Of the 12 patients with an apical systolic murmur, 4 had left ventricular enlargement; however, each of them also had concomitant aortic insufficiency. A total of 6 patients (Nos. 4, 6, 16, 18, 19, and 20) had aortic insufficiency, and all but 2 of these (Nos. 16 and 18) had slight to moderate left ventricular enlargement.

Right heart catheterization was performed with each patient in the postabsorptive state. The catheter (single or double lumen) was passed into the pulmonary artery. In some cases it was then advanced into the peripheral lung field where a resting "pulmonary venous capillary" ("PVC") pressure was recorded.[24, 25] In attempting to record "PVC" pressures far out in the pulmonary arterial tree, one frequently obtains damped pulmonary artery pressure curves. Although one may be able with suction to draw a blood sample fully saturated with oxygen at such a site, this cannot be considered as adequate evidence in any particular case that the pressure recorded without the suction at the same site is a "PVC" pressure. If the vessel is incompletely occluded by the catheter during any phase of the cardiac cycle, the pressure will not be truly "PVC," but may represent a mixture of both pulmonary artery and "PVC" pressures. At the present time, we consider a pressure curve, taken in a peripheral pulmonary artery, to be representative of the "PVC" pressure only when the curve recorded shows multiple oscillations in diastole as well as systole, has no evidence of damping, and does not resemble a pulmonary artery pressure curve.

From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital. This Department is supported in part by the Michael Reese Research Foundation.
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TABLE I. DATA ON OXYGEN CONSUMPTION, A-V OXYGEN DIFFERENCE, CARDIAC AND STROKE OUTPUTS IN PATIENTS WITH MITRAL STENOSIS AT REST AND WITH EXERCISE

CASE NO.	AGE (YR.)	RHYTHM	L.V.	O_2 CONSUMPTION (C.C./MIN./M²)		A-V OXYGEN DIFFERENCE (VOL. PER CENT)		BRACH. ART. SATURATION (PER CENT)		CARDIAC OUTPUT (L./MIN.)		CARDIAC INDEX (L./MIN./M²)		PULSE RATE (BEATS/MIN.)		STROKE INDEX (ML./BEAT/M²)	
				REST	EX.	REST	EX.	REST	EX.	REST	EX.	REST	EX.	REST	EX.	REST	EX.
1	34	R	N	133	249	4.8	6.5	96	–	4.5	6.2	2.8	3.8	90	–	31	–
2	22	R	N	143	370	5.0	9.4	94	95	3.9	5.4	2.9	3.9	107	138	27	28
3	21	R	N	106	236	3.1	5.5*	96	–	5.2	6.6*	3.1	4.3*	73	95	42	45*
4	45	F	E–	119	295	6.9	11.8	92	95	2.7	3.9	1.7	2.5	70	157	24	16
5	22	F	N	140	–	6.7	11.0	98	97	3.9	4.9	2.2	2.6	71	124	31	21
6	23	F	E	185	423	4.1	9.6	96	99	8.4	8.2	4.5	4.4	90	135	50	33
7	38	R	N	126	415	3.7	8.6	95	100	5.9	8.4	3.4	4.8	72	106	47	45
8	33	R	N	119	306	4.6	10.2	97	100	3.7	4.4	2.6	3.0	75	130	35	23
9	53	F	N	136	294	4.6	8.3	95	94	5.1	6.2	2.9	3.5	88	120	33	29
10	39	R	N	143	–	5.3	–	94	–	4.0	–	2.7	–	82	–	33	–
11	44	R	N	135	439	4.6	8.9*	99	–	5.3	9.1*	2.9	5.0*	83	130	35	38*
12	42	F	N	101	285	6.4	12.1	100	98	3.0	4.5	1.6	2.4	50	103	32	23
13	47	F	N	–	–	–	–	–	–	–	–	–	–	80	–	–	–
14	35	R	N	165	–	7.6	–	90	90	3.9	–	2.2	–	100	–	22	–
15	30	R	N	105	326	5.0	7.7	99	99	3.3	6.7	2.1	4.2	60	108	35	39
16	19	R	N	117	274	3.8	5.4	100	100	4.8	7.9	3.1	5.1	75	100	41	51
17	42	R	N	104	252	4.5	6.9	96	95	3.7	5.8	2.3	3.7	90	102	26	36
18	32	F	E	115	290	7.9	14.2	96	97	2.6	3.6	1.5	2.1	65	120	23	18
19	31	R	E	128	331	4.2	8.1	92	92	5.6	7.6	3.1	4.1	88	115	35	36
20	41	F	N	104	270	5.1	9.2	97	97	3.3	4.8	2.0	2.9	60	92	33	32
Average 35				128	317	5.2	9.0	96	96	4.4	6.1	2.6	3.7	78	117	33	32

*Estimated.

R = Regular sinus rhythm; F = auricular fibrillation; L.V. = left ventricle; N = normal on x-ray or fluoroscopy; E = enlarged on x-ray or fluoroscopy; Ex. = exercise; Brach. Art. = brachial artery

Afterward, the catheter was withdrawn to the main pulmonary artery or near the bifurcation of the main pulmonary artery. After waiting for stabilization of the patient's pulse and respirations, the resting cardiac output was obtained. Expired air was collected in a Tissot spirometer for a five-minute period, and resting pressures were obtained during this time. Brachial artery blood samples and pressures were obtained through an indwelling arterial needle. Simultaneous samples from the brachial artery and pulmonary artery were taken during the third minute of the resting gas collection. Following the resting period, exercise was performed for five minutes (except for patients 5 and 14 who exercised for three minutes, and patient 1 who exercised for six minutes) in the recumbent position by pedaling a bicycle wheel (pedal radius, 8 cm.; wheel radius, 23 cm.) at a steady rate of approximately 80 turns a minute against a fixed resistance of 500 Gm. Blood samples and pressures were obtained during the fourth and fifth minutes of the exercise period. Expired gases were collected in Douglas bags during the first, second and third, and fourth and fifth minutes of the exercise, as well as during the first and last four minutes of a five-minute recovery period. The oxygen consumption during the last two minutes of exercise was used in calculating the "exercise cardiac output."[34]

The zero level for all pressures was taken as 5 cm. below the angle of Louis. Pressures were taken with capacitance type electromanometers* recording on a direct-writing polyoscillograph.* Mean pressures were obtained by electrical or planimetric integration. The pressure recordings were calibrated with a mercury manometer. Expired air samples were analyzed for carbon dioxide on the Haldane-Henderson apparatus and for oxygen on the Pauling analyzer. The volume of oxygen consumed is expressed as dry gas at 0° C. and 760 mm. Hg pressure. Blood oxygen analyses were performed according to the method of Van Slyke and Neill.[21] Cardiac outputs were calculated in accordance with the Fick principle. So-called pulmonary arteriolar resistance was calculated by the method of Gorlin and associates.[15]

We determined the work of the right ventricle in terms of work per minute per square meter of body surface in accordance with formulas previously developed in this department.[22, 23] When considering normal patients, little error is introduced by failing to subtract the mean right auricular (or right ventricular end diastolic) pressure from the mean pulmonary artery pressure. In calculating the right ventricular work in the present study, the mean right auricular (or right ventricular end diastolic) pressure, which was often elevated at rest or during exercise, was subtracted from the mean pulmonary artery pressure. In those cases where the mean auricular (or right ventricular end diastolic) pressure was not determined during exercise, the resting value was subtracted from the mean pulmonary artery pressure during exercise before computing the exercise right ventricular work.

No effort was made to determine the kinetic energy because of the difficulties involved.[22] Right ventricular work was calculated in terms of potential energy only. This is not an important omission in these patients because the principal factors determing the kinetic energy (stroke volume and caliber of pulmonary artery) tend, in patients with mitral stenosis, to make the kinetic energy value small even with exercise.[23]

RESULTS

In Table I and Fig. 1 it is shown that the cardiac index at rest in these patients was generally reduced. In eight patients it was reduced below the lower limits of the normal resting cardiac index of 2.6 L. per minute per square meter. In several of the others it was at the lower range of normality. The average resting cardiac index for the entire group was 2.6 L. per minute per square meter. Since the pulse rate in most of the patients was normal or slightly elevated, the stroke output was generally decreased. The average resting stroke index was 33 c.c. per beat.

*Sanborn.

There was a rise in cardiac output on exertion, although often slight, in sixteen out of seventeen of the patients in whom the determination was made (Table I; Fig. 1). The average cardiac index with exercise was 3.7 L. per minute per square meter. This is relatively lower, compared with the normal, than the resting cardiac index. Consequently, the slope of the lines connecting resting and exercise $\frac{\text{oxygen consumption}}{\text{cardiac index}}$ ratio is, as expected, generally steeper in the mitral patients than in the average normal.

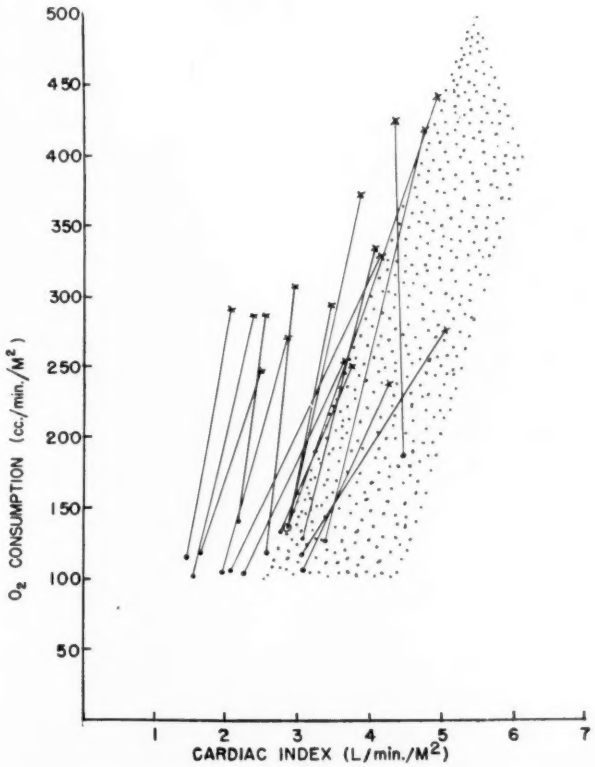

Fig. 1.—The correlation of the oxygen consumption and the cardiac index at rest (●) and exercise (×) in 17 patients with mitral stenosis. The resting and exercise values are connected by straight lines. The stippled area defines the limits of normal values at rest and exercise as determined in normal individuals studied in this laboratory and normals reported by others.[12, 26, 39]

"Pulmonary venous capillary" ("PVC") pressures[24, 25] were obtained, at rest only, in 10 patients, and were found to be elevated above the upper normal limit of 12 mm. Hg[25, 26] in six. In 3 patients in whom "PVC" pressures were obtained (patients 4, 7, and 8) and in one patient in whom unsatisfactory pressures were obtained (patient 13), there was evidence on the day following catheterization of pulmonary infarction (localized râles and hemoptyses in all 4, roentgenological evidence in 3). The site of infarction was always in the area of the lung where the "PVC" pressure was taken. No patient in whom a "PVC" pressure was not taken showed any signs of pulmonary in-

farction after catheterization, although the procedures used were otherwise identical. Furthermore, "PVC" pressures have been taken in this department in approximately 40 patients with various forms of cardiopulmonary disorders, but without mitral stenosis, and in none was there any evidence of pulmonary infarction.

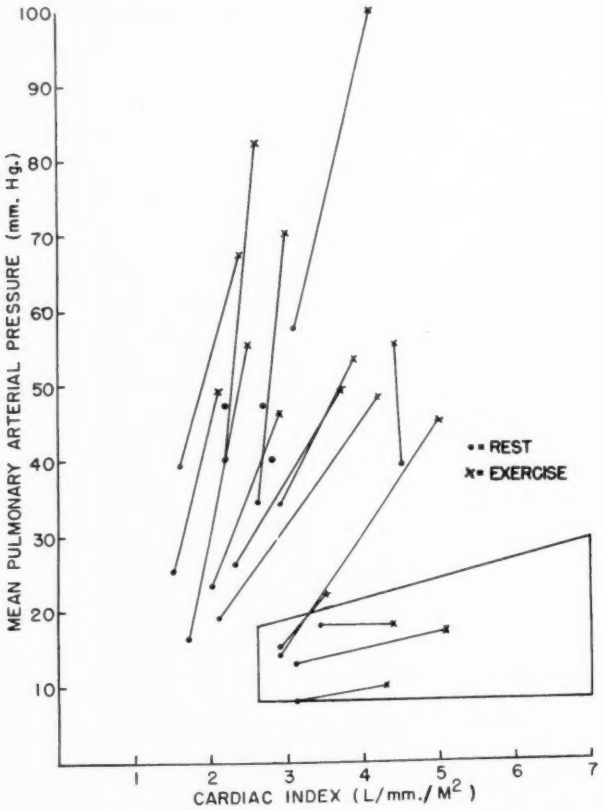

Fig. 2.—The correlation of mean pulmonary artery pressure and cardiac index in patients with mitral stenosis at rest and during exercise. Conventions as in Fig. 1. The trapezoid encloses the normal range for these values.[12, 26]

In Table II and Fig. 2 are shown the pressure data from the pulmonary artery. In 6 patients the mean pulmonary artery pressure at rest was within normal limits (up to 18 mm. Hg).* In 3 of these 6 cases the pulmonary artery pressure remained normal with exercise, while in the other 3 patients it rose to abnormally high levels. In the remaining 14 patients, the pulmonary artery pressure was elevated at rest and rose still higher in those who were exercised.

There was evidence of right ventricular failure at rest (end diastolic pressure greater than 6 mm. Hg)* in 3 patients (see Table II). Pressures in the right ventricle during exercise were determined in 9 patients. One of these 9 patients had right heart failure at rest, and further elevation of the right

*These normal values are based on the findings in normal individuals studied in this laboratory and reports in the literature.[12, 22, 26, 27]

ventricular end diastolic pressure occurred with exercise. Four of the other 8 patients with normal resting end diastolic pressures developed right ventricular failure, as evidenced by abnormally high end diastolic pressures, with exercise (patients 15, 17, 19, and 20).

The calculated so-called resting pulmonary arteriolar resistance (according to the criteria of Dexter and associates) in 9 of 10 patients in whom a "PVC" pressure was obtained, was abnormally elevated (Table II; Fig. 3).

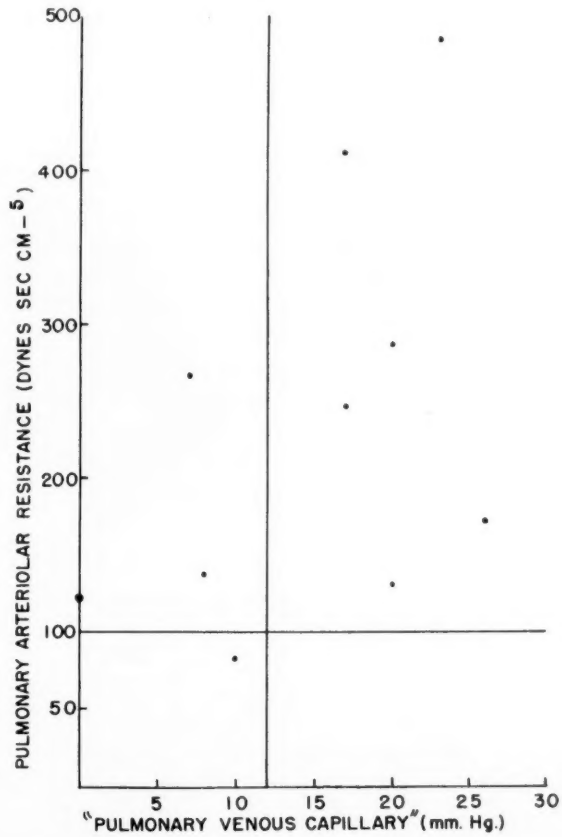

Fig. 3.—The correlation of "PVC" pressure and pulmonary arteriolar resistance calculated on the assumptions made and formula used by Gorlin and associates.[15] The vertical and horizontal lines represent the upper range of normal for "PVC" pressure and pulmonary arteriolar resistance, respectively.

At rest, the oxygen consumption varied from 100 to 185 ml./min./M² body surface with only 2 patients having oxygen consumptions greater than 150 ml./min./M² (Table I). The patient with the greatest oxygen consumption (patient 6) was decidedly anxious at the time of the determination, and this was probably responsible for the high value obtained. The oxygen consumption with exercise in the 16 patients in whom exercise was done varied from 225 ml./min./M² to 440 ml./min./M² (Table I). We used the oxygen consumption increase as an index of the effort involved in the exercise. Thus the effort in

TABLE II. DATA ON PRESSURES, PULMONARY ARTERIOLAR RESISTANCE AND RIGHT VENTRICULAR WORK IN PATIENTS WITH MITRAL STENOSIS

CASE NO.	PVC (REST) (MM. HG)	MEAN PULMONARY ARTERY (MM. HG)		MEAN BRACHIAL ARTERY (MM. HG)		SYST./DIAST. PRESSURE RIGHT VENTRICLE (MM. HG)		RESTING "PULMONARY ARTERIOLAR RESISTANCE" (DYNES SEC. CM^{-5})	WORK OF RIGHT VENTRICLE (KG. M./MIN./M^2)	
		REST	EX.	REST	EX.	REST	EX.		REST	EX.
1	17	40	--	--	--	50/0	--	411	1.5	--
2	20	34	53	65	76	44/0	--	287	1.3	2.8
3	0	8	10	85	90	14/–1	--	123	0.38	0.7
4	7	16	55	112	--	28/0	60/0	266	0.37	1.9
5	--	40	82	96	--	55/4	110/4	--	1.1	2.8
6	--	39	55	100	120	61/5	--	--	2.1	3.0
7	8	18	18	82	--	30/6	--	136	0.56	0.78
8	26	34	70	82	--	42/8	--	172	0.92	2.5
9	10	15	22	80	--	22/0	33/3	78	0.59	0.90
10	23	47	--	82	--	87/5	--	485	1.5	--
11	--	14	45	--	--	30/3	--	--	0.43	2.9
12	--	39	68	90	--	46/8	88/18	--	0.68	1.6
13	--	25	--	--	--	30/7	--	--	--	--
14	--	47	--	72	--	78/2	--	--	1.3	--
15	--	19	48	82	--	29/2	60/17	--	0.49	1.8
16	--	13	17	78	82	20/2	--	--	0.46	1.0
17	20	26	49	70	--	40/2	64/9	131	0.75	2.0
18	17	25	49	87	--	32/1	60/–1	246	0.49	1.4
19	--	57	100	73	--	77/2	127/9	--	2.3	5.1
20	--	23	46	88	--	30/5	58/10	--	0.49	1.4
Average		29	49	84		43/3	73/8	234–	0.93	2.0

PVC = "Pulmonary venous capillary"; Ex. = exercise; Syst. = systolic; Diast. = diastolic.

the patients during exercise on this basis varied from 187 per cent to 329 per cent of their effort at rest. The exercise demanded was deliberately mild in order that it could be performed by all patients. The effective amount of external work performed by the patient in pedalling a bicycle wheel was similar in all cases. However, as expected in untrained subjects, there was considerable variability in the efficiency with which such external work was performed.

In 7 of 19 patients, the right ventricular work was increased at rest (Table II). The average resting right ventricular work of the 19 patients was 0.93 Kg.M./min./M^2, while the average right ventricular work during exercise (16 patients) was 2.0 Kg.M./min./M^2. In the few normals reported in the literature with this range of exercise,[12, 26] the average right ventricular work performed was 0.60 Kg.M./min./M^2 at rest and 1.3 Kg.M./min./M^2 during exercise.

DISCUSSION

Our observations on cardiac output at rest and exercise are in accord with the work of Meakins and associates,[9] using a modification of Haldane's carbon dioxide method, Stewart and associates,[11] using the acetylene method, and many recent studies[12-19] using the technique of right heart catheterization. They also show a variable range of effect, as reported by others. It is clear that mitral stenosis has a variable tendency to reduce the resting cardiac output. Its effect in this direction during exercise is more striking.

The increased oxygen requirements of the body during exercise in normal individuals are provided by an elevated cardiac output which increases the amount of oxygen reaching the tissues and by an increased oxygen removal from the blood in the capillaries. When the cardiac output can increase without restriction, and especially in trained individuals, it suffices to meet demands without undue increase in oxygen removal from the capillaries. The latter will increase abnormally whenever the cardiac output adjustment is restricted. While the normal individual can provide the tissue oxygen needs during exercise, as a rule, primarily by an increased blood flow, patients with mitral stenosis are able to increase their cardiac output to a limited extent only. This is shown in Fig. 1, where the relationship between the oxygen consumption and cardiac index is plotted and compared with the average normal response. With this limited cardiac output, the tissue oxygen requirements are satisfied to a greater extent than in the normal by an increased oxygen uptake from the capillary blood, thus resulting in an abnormally large arteriovenous oxygen difference. This abnormally large A-V oxygen difference in patients with mitral stenosis may be present at rest (because of reduced cardiac output) as well as during exercise, but the deviation from the normal is generally more marked during exercise. The magnitude of the A-V oxygen difference serves as an excellent guide as to the patient's ability to increase his cardiac output adequately during exercise.[12] In general, the greater the A-V oxygen difference for a given amount of exercise, the less adequate is the cardiac output in supplying the tissue oxygen requirements. This is shown in Fig. 4. While 13 of the mitral patients had normal A-V oxygen differences at rest, only 4 remained in the normal range during exercise (patients 1, 3, 16, and 17).

This limited rise in cardiac output in patients with mitral stenosis has been observed previously. In fact, some patients with mitral stenosis have shown a decreased cardiac output during exercise (13, 17, and 19) in spite of the fact that the pressure gradient across the mitral orifice probably rises and would tend to increase blood flow into the left ventricle. The explanation for the limited response to exercise is not completely clear, but several factors seem to play significant roles.

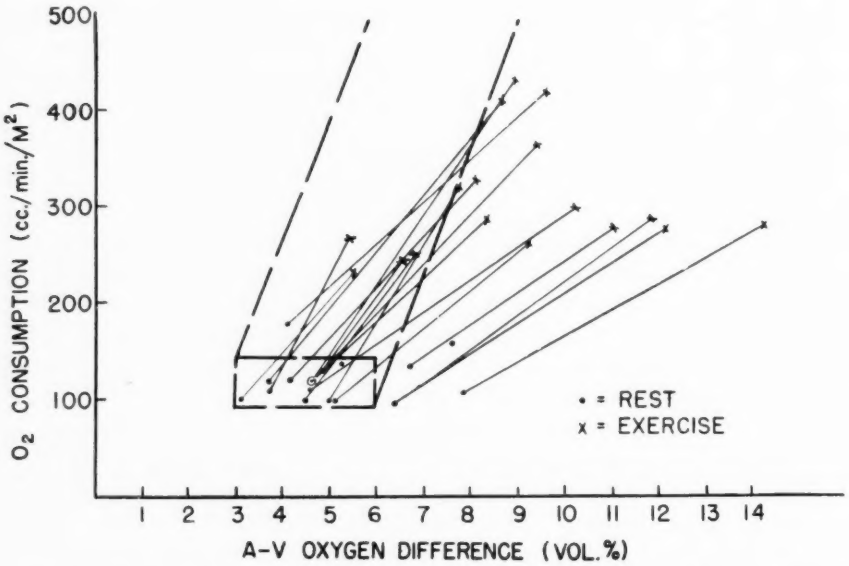

Fig. 4.—The correlation of oxygen consumption and A-V oxygen difference in patients with mitral stenosis at rest and exercise. The resting and exercise values, where exercise was performed, are connected by straight lines. Conventions as in Fig. 1. The rectangle and the straight lines from the extremities of the rectangle enclose the range of normal values found at rest and during exercise in normal individuals studied in this laboratory and reported by others.[12, 26, 39, 40] Figure adapted from Hickam and Cargill.[12]

The possibility of some left ventricular failure with exercise in patients with mitral stenosis cannot be completely eliminated, since diastolic left ventricular pressures have not been determined in these patients. It is well known that patients in left heart failure may show little or no increase (or even a decrease) in cardiac output with exercise. Hickam and Cargill[12] noted the similarity in the response to exercise of patients with mitral stenosis and left ventricular failure (increased pulmonary artery pressure, increased A-V oxygen difference, and limited cardiac output response). Left ventricular failure may be a factor in those patients with significant regurgitation at the mitral and aortic valves. It seems unlikely, however, that such a mechanism plays a significant role in limiting the increase in cardiac output during exercise in patients with isolated mitral stenosis. Most rheumatic patients with mitral stenosis as the predominant valvular lesion show little evidence of left ventricular disease.[28] Seven of our patients who had an abnormal A-V oxygen difference with exercise had "pure" mitral stenosis with no evidence of left ventricular disease.

The possibility exists that right ventricular failure might develop in mitral patients during exercise, as a result of the increased work of the right ventricle against pressure. This was investigated by analysis of the right ventricular end diastolic pressure. There was evidence of right ventricular failure during exercise with abnormal elevation in the end diastolic pressure in 5 of 9 patients in whom the right ventricular pressures were taken. In some cases therefore, this failure of the right ventricle during exercise may play a significant part in restricting the cardiac output.

In the presence of mitral stenosis, the possibility exists that significant left ventricular filling occurs throughout diastole, and thus the more rapid heart rate occurring during exercise may eliminate a significant part of this inflow. In the normal heart, left ventricular filling is largely confined to the rapid inflow phase occurring early in diastole.[29] Moderate acceleration of the heart would thus interfere only slightly with filling and would have, therefore, little effect on stroke volume. Minute volume, the product of stroke volume and heart rate, would be greatly increased normally as a consequence. With rapid inflow more spread out, as is postulated in mitral stenosis, the effect of cardiac acceleration on filling and stroke volume would be enhanced, and hence the minute output augmentation would be reduced.

If mitral insufficiency of significant degree is also present, a more rapid heart rate would give more opportunity for regurgitation. This is so because as the heart speeds up, more of its time is spent in systole, the phase of the heart cycle during which regurgitation would take place. This is based on the fact that systole shortens much less than diastole as the rate accelerates and systolic time is the product of the heart rate and the duration of systole.

More significant than these factors, it would appear, is the limitation on cardiac output imposed by a tight mitral orifice per se. It has been shown that the dynamic effect of mitral stenosis is not linearly proportional to its degree, but tends to be curvilinear,[30, 31] i.e., each increment of stenosis has a greater and greater effect than the preceding increment. This means that as the stenosis becomes tighter, progressively greater and greater pressure differences are needed across the orifice to produce equivalent increases in flow. Or, contrariwise, an equivalent pressure gradient increase will have progressively less and less effect on flow until ultimately the effect is negligible. This is the most important cause for the diminished or absent augmentation of cardiac output on exercise with mitral stenosis. It is for this among other reasons that the term "dynamic significance" of mitral stenosis was created. Thus in dynamically significant mitral stenosis, the limited egress of blood from the lungs on exercise, with a comparatively normal input by the right heart, sets up conditions for pulmonary engorgement and its sequelae.

All of our patients with mitral stenosis had an increase in cardiac output (though generally small) with exercise, with one exception (patient 6). This patient, however, manifested a good deal of anxiety during the catheterization procedure, as is evidenced by this high resting oxygen consumption and elevated cardiac output. The dissipation of his anxiety during exercise may have been

responsible for the failure of this patient to increase his cardiac output above that present when he was "anxiously resting." Hickam and associates[32] have noted that normal patients may show a drop in cardiac output in going from a state of anxiety to exercise. This mechanism may be responsible for the occasional decrease in cardiac output on mild exercise in cases with mitral stenosis reported by others.

Another possible reason for failure to observe an increase in cardiac output on exercise in mitral stenosis patients lies in the difficulty in obtaining a "steady state" of exercise. Thus during short periods of exercise of three minutes or less[17, 18] there may be fluctuations in oxygen consumption and A-V oxygen difference. This arises because of the difficulty in obtaining arterial and venous blood samples precisely representative of the changing blood gas pattern and because the oxygen consumption may still fluctuate early in exercise.[33, 34] We have attempted to decrease this source of error by measuring the oxygen consumption and obtaining our blood samples during the fourth and fifth minutes of exercise when things are steadier; even at this time, however, a steady state need not always be present.[34] Finally, the lack of increase in cardiac output during exercise in some of the reported cases may be due to the extreme mildness of the exercise itself which was employed by others.[17]

We were unable to confirm the findings of Stewart and his co-workers[11] (using the acetylene method) that impairment of cardiac output was generally less in those patients who had aortic insufficiency in addition to their mitral valve disease than in patients with isolated mitral valve lesions. The six patients (patients 4, 6, 16, 18, 19, and 20) in the present series who had aortic insufficiency had catheterization findings essentially similar in all respects to the remainder of the group, at rest and on exercise. The findings of Stewart and associates may be the result of fortuitous sampling or to inadequacies of the acetylene method employed.

Only three of our patients (patients 4, 14, and 19*) showed arterial unsaturation. The rest, like the majority reported in the literature, showed normal oxygen saturation of the peripheral arterial blood. On occasion, however, the pathologic changes in the lung associated with mitral stenosis[35, 36] may produce inadequate pulmonary gas exchange and thus lead to arterial unsaturation. None of our three patients with arterial oxygen unsaturation had evidence of pulmonary edema at the time of cardiac catheterization. It is assumed, therefore, that in them pathologic alterations in the alveoli and small pulmonary vessels were responsible for the unsaturation of the arterial blood. Cyanosis, however, when it occurs in patients with mitral stenosis, is generally due to stasis in the periphery of the systemic circuit.[37]

The apparent increase in pulmonary arteriolar resistance at rest found in 4 of our 9 patients in whom it was measured, on the basis of certain assumptions,[15] has been suggested previously in patients with mitral stenosis.[15, 17, 19] There are sufficient pathologic changes in the pulmonary arteriolar, in addition

*In patient 19, biopsy specimens taken at the time of surgery showed the presence of miliary granulomatosis, consistent with sarcoidosis, in the pleura, lung, pericardium and left auricular appendage.

to the arterial, tree to account for an increased resistance. The possibility that active pulmonary arteriolar constriction is an important factor is unlikely, but has not been eliminated.

The exact etiology for the episodes of pulmonary infarction which occurred in 4 patients following catheterization is uncertain. The episodes do seem to bear a relation to the procedure used to measure "PVC" pressures. Signs of pulmonary infarction developed eight to thirty-six hours after catheterization in 4 of 10 mitral stenosis patients in whom efforts to take "PVC" pressures were made, but in none of the other patients. Furthermore, the infarction in each case occurred in precisely the area where the "PVC" pressure was obtained (right middle lobe in two patients, right lower lobe in the others). Although Hellems and his co-workers[24] noted no untoward consequences of "PVC" measurements in dogs, it is possible that in our patients the catheter tip plugging the pulmonary arteriole caused injury to the intima, and this in turn may have been followed by extensive thrombosis of the injured artery. The presence of pulmonary vascular disease in patients with mitral stenosis may account for a disposition to injury and thrombosis. Pulmonary congestion, which is common in mitral stenosis, would contribute to the infarction of the lung. With pulmonary congestion, protracted obstruction of the smaller pulmonary artery branch itself could conceivably lead to infarction without injury or thrombosis of the vessel. Dexter[38] states that he has been able to eliminate this complication by sterilization of the catheters by autoclaving. The fact that in two of our cases the "PVC" pressure was not elevated at rest may not be used as proof of absence of congestion. We think it highly unlikely that pulmonary embolism could have caused pulmonary infarction in these 4 patients and involve in each case the exact area supplied by the pulmonary artery branch obstructed. At present, when we obtain "PVC" pressures, we permit the catheter to plug the artery for seconds only and we do not take these pressures during exercise. We recommend such precautions to all workers in this field.

Our primary purpose in performing cardiac catheterization studies in patients with mitral stenosis was to define the variable dynamic significance of this lesion in individual cases, with a view to possible surgical relief of the stenosis. Reliable criteria for the precise definition of surgical indications and contraindications can be obtained only by careful analysis of such physiological data collected on a large number of individuals before and after surgery, as well as on patients who are not operated upon. At the present, only tentative opinions may be entertained in regard to surgery on the basis of a limited number of observations.

On the basis of clinical and physiological studies, a mitral valve stenosis may be classified as being mild, moderate, or severe (Fig. 5). It is the moderate and severe stenoses that we consider "dynamically significant." Furthermore, the valve lesion must be considered from the aspect of whether it is or is not progressive.

The selection of patients with mitral stenosis for valvulotomy requires considerable thought and study devoted to each patient. The proper decision in many cases will depend not only on special cardiovascular studies but also will require careful and repeated evaluation of each patient with valvular heart

disease by the internist or cardiologist to decide upon the advisability of surgery. The responsibility for choosing the appropriate time for surgery rests primarily with the internist and cardiologist. It is often an extremely difficult task and must be faced with considered and skilled judgment if the best medical and surgical results are to be realized.

SUMMARY

Cardiovascular dynamics were studied in 20 patients with mitral stenosis by means of right heart catheterization.

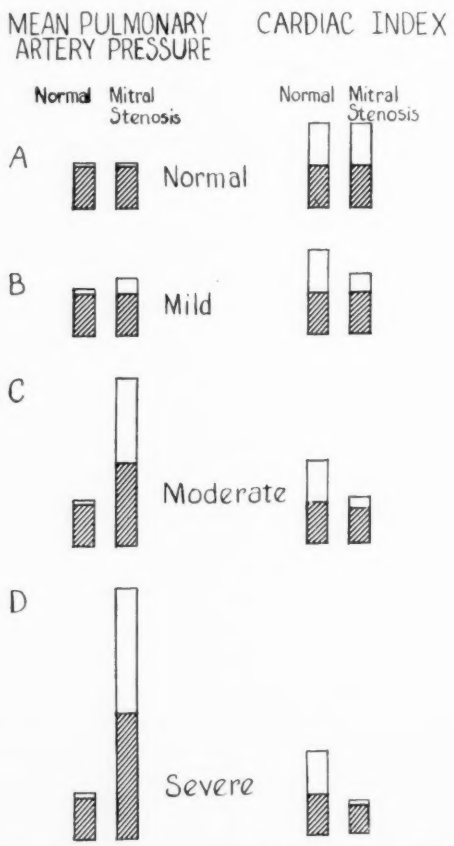

Fig. 5.—This is a diagrammatic representation of the effect of different degrees of mitral stenosis on mean pulmonary artery pressure and cardiac index at rest and on exercise as compared to paired normals. The pairs of blocks on the left represent mean pulmonary artery pressure. The pairs of blocks on the right represent cardiac index. In each pair, the block to the left is the normal subject, to the right the subject with mitral stenosis. The height of the shaded portion of the block represents in each case the mean pulmonary artery pressure and cardiac index at rest, and the total height represents these values on exercise. The unshaded portion of the block thus represents the effect of exercise. *Row A*, Dynamically insignificant mitral stenosis. The mean pulmonary artery pressure and cardiac index are normal at rest and during exercise. *Row B*, Mild mitral stenosis. The pulmonary artery pressure and cardiac index are normal at rest. On exercise, however, the pulmonary artery pressure rises excessively while the rise in cardiac index is less than normal. *Row C*, Moderate mitral stenosis. The resting pulmonary artery pressure is elevated and the resting cardiac index is reduced. On exercise, there is a marked rise in pulmonary artery pressure while the cardiac index increases very little. *Row D*, Severe mitral stenosis. There is a marked pulmonary artery hypertension at rest while the resting cardiac index is low. On exercise, there is an extreme rise in pulmonary artery pressure while there is insignificant increase in cardiac index.

At rest there was generally an increased mean pulmonary artery pressure, a reduced cardiac output, an increased "PVC" pressure, and an increased "pulmonary arteriolar" resistance.

Generally there was only a small rise in cardiac output on exercise associated with a marked rise in pulmonary artery pressure. The possible mechanisms for the limited cardiac output response on exercise in patients with mitral stenosis are discussed.

There was evidence of pulmonary infarction in 4 of 10 patients eight to thirty-six hours after "PVC" pressures were taken at rest. The possible etiology of the infarcts is discussed.

On the basis of preliminary observations, surgery is indicated in dynamically significant stenosis and when the disease appears to be progressive, unless some specific contraindication is present.

In view of the beneficial effects frequently observed after valvulotomy in patients with mitral stenosis, every patient with this lesion deserves particularly careful and frequent evaluation. The data obtained by cardiac catheterization studies are of great aid in such evaluation.

We wish to acknowledge our appreciation to the other members of the catheterization and respiratory teams for technical aid. We are indebted to the physicians of Michael Reese Hospital for permitting us to study their patients. We would like to express our thanks also to those physicians who attended the special weekly conferences where each case was discussed, and who contributed to the thoughts expressed in this paper.

REFERENCES

1. Cutler, E. C., and Levine, S. A.: Cardiotomy and Valvulotomy for Mitral Stenosis, Boston M. & S. J. **188:** 1023, 1923.
2. Allen, D. S.: Intracardiac Surgery, Arch. Surg. **8:** 317, 1924.
3. Souttar, H. S.: The Surgical Treatment of Mitral Stenosis, Brit. M. J. **2:** 603, 1925.
4. Cutler, E. C.: The Surgical Aspect of Mitral Stenosis, Arch. Surg. **12:** 212, 1926.
5. Bailey, C. P., Glover, R. P., and O'Neill, T. J. E.: The Surgery of Mitral Stenosis, J. Thoracic Surg. **19:** 16, 1950.
6. Harken, D. E., Ellis, L. G., Ware, P. F., and Norman, L. R.: The Surgical Treatment of Mitral Stenosis: I. Valvuloplasty, New England J. Med. **239:** 801, 1948.
7. Smithy, H. G., Boone, J. A., and Stallworth, J. M.: Surgical Treatment of Constrictive Valvular Disease of the Heart, Surg., Gynec. & Obst. **9:** 175, 1950.
8. Baker, C., Brock, R. C., and Campbell, M.: Valvulotomy for Mitral Stenosis: Report of 6 Successful Cases, Brit. M. J. **1:** 1283, 1950.
9. Meakins, J. C., D'Autrebande, L., and Fetter, W. J.: The Influence of Circulatory Disturbances on the Gaseous Exchange of the Blood: IV. The Blood Gases and Circulation Rate in Cases of Mitral Stenosis, Heart **10:** 153, 1923.
10. Kerkhof, A. C.: Minute Volume Determinations in Mitral Stenosis During Auricular Fibrillation and After Restoration of Normal Rhythm, Am. Heart J. **11:** 206, 1936.
11. Stewart, H. J., Deitrick, J. E., Watson, R. F., Wheeler, C. H., and Crane, N. F.: The Effect of Valvular Heart Disease on the Dynamics of the Circulation, Am. Heart J. **16:** 477, 1938.
12. Hickam, J. B., and Cargill, W. H.: Effects of Exercise on Cardiac Output and Pulmonary Arterial Pressure in Normal Persons and in Patients with Cardiovascular Disease and Pulmonary Emphysema, J. Clin. Investigation **27:** 10, 1948.
13. Ellis, L. B., and Harken, D. E.: Mitral Stenosis, Clinico-Physiologic Correlations with Particular Reference to Surgical Intervention, Tr. Am. Clin. Climatol. Assoc. **60:** 59, 1948.
14. Borden, C. W., Ebert, R. V., Wilson, R. H., and Wells, H. S.: Pulmonary Hypertension in Heart Disease, New England J. Med. **242:** 529, 1950.
15. Gorlin, R., Haynes, F. W., Goodale, W. T., Sawyer, C. G., Dow, J. W., and Dexter, L.: Studies of the Circulatory Dynamics in Mitral Stenosis: II. Altered Dynamics at Rest, Am. Heart J. **41:** 30, 1951.
16. Gorlin, R., Sawyer, C. G., Haynes, F. W., Goodale, W. T., and Dexter, L.: Effects of Exercise on Dynamics in Mitral Stenosis: III, Am. Heart J. **41:** 192, 1951.

17. Draper, A., Heimbecker, R., Daley, R., Carroll, D., Mudd, G., Wells, R., Falholt, W., Andrus, E. C., and Bing R. J.: Physiologic Studies in Mitral Valvular Disease, Circulation **3**: 531, 1951.
18. Ellis, L. B., Bloomfield, R. A., Graham, G. K., Greenberg, D. J., Hultgren, H. N., Kraus, H., Maresh, G., Mebane, J. G., Pfeiffer, P. H., Selverstone, L. A., and Taylor, J. A.: Studies in Mitral Stenosis: I. A Correlation of Physiologic and Clinical Findings, Arch. Int. Med. **88**: 515, 1951.
19. Bayliss, R. I. S., Etheridge, M. J., and Hyman, A. L.: Pulmonary Hypertension in Mitral Stenosis, Lancet **259**: 889, 1950.
20. Nomenclature and Criteria for Diagnosis of Diseases of the Heart, New York Heart Association, 1939.
21. Van Slyke, D. D., and Neill, J. M.: Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, J. Biol. Chem. **61**: 523, 1924.
22. Prec, O., Katz, L. N., Sennett, L., Rosenman, R. H., Fishman, A. P., and Hwang, W.: Determination of Kinetic Energy of the Heart in Man, Am. J. Physiol. **159**: 483, 1949.
23. Landowne, M., and Katz, L. N.: Heart; Work and Failure, Medical Physics. Vol. 2. Chicago, 1950, Year Book Publishers.
24. Hellems, H. K., Haynes, F. W., Dexter, L., and Kinney, T. D.: Pulmonary Capillary Pressure in Animals Estimated by Venous Catheterization, Am. J. Physiol. **155**: 98, 1948.
25. Hellems, H. K., Haynes, F. W., and Dexter, L.: Pulmonary "Capillary" Pressure in Man, J. Applied Physiol. **2**: 24, 1949.
26. Dexter, L., Whittenberger, J. L., Haynes, F. W., Goodale, W. J., Gorlin, R., and Sawyer, C. G.: Effect of Exercise on Circulatory Dynamics of Normal Individuals, J. Applied Physiol. **3**: 439, 1951.
27. Bloomfield, R. A., Lauson, H. D., Cournand, A., Breed, E. S., and Richards, D. W., Jr.: Recording of Right Heart Pressures in Normal Subjects and in Patients with Chronic Pulmonary Disease and Various Types of Cardio-Circulatory Disease, J. Clin. Investigation **25**: 639, 1946.
28. Walsh, B. J., Bland, E. F., and Jones, T. D.: Pure Mitral Stenosis in Young Persons, Arch. Int. Med. **65**: 321, 1940.
29. Wiggers, C. J.: Studies on the Consecutive Phases of the Cardiac Cycle: I. The Duration of the Consecutive Phases of the Cardiac Cycle and the Criteria for their Precise Determination, Am. J. Physiol. **56**: 415, 1921.
30. Gorlin, R., and Gorlin, S. G.: Hydraulic Formula for Calculation of the Area of the Stenotic Mitral Valve, Other Cardiac Valves, and Central Circulatory Shunts: I, Am. Heart J. **41**: 1, 1951.
31. Katz, L. N., and Siegel, M. L.: Cardiodynamic Effects of Acute Experimental Mitral Stenosis, Am. Heart J. **6**: 672, 1931.
32. Hickam, J. B., Cargill, W. H., and Golden, A.: Cardiovascular Reactions to Emotional Stimuli. Effect on the Cardiac Output, Arteriovenous Oxygen Difference, Arterial Pressure and Peripheral Resistance, J. Clin. Investigation **27**: 290, 1948.
33. Buck, A. V., Vancaulaert, C., Dill, D. B., Folling, A., and Hurxthal, L. M.: Studies in Muscular Activity: IV. The "Steady State" and the Respiratory Quotient During Work, J. Physiol. **66**: 162, 1928.
34. Snider, G. L., Elisberg, E. I., Miller, G., Radner, D., and Katz, L. N.: Cardiopulmonary Studies in Patients with Mitral Stenosis. II. Pulmonary Function Studies, In preparation.
35. Parker, F., Jr., and Weiss, S.: The Nature and Significance of the Structural Changes in the Lungs in Mitral Stenosis, Am. J. Path. **12**: 573, 1936.
36. Larrabee, W. F., Parker, R. L., and Edwards, J. E.: Pathology of Intrapulmonary Arteries and Arterioles in Mitral Stenosis, Proc. Staff Meet. Mayo Clin. **24**: 316, 1949.
37. Cossio, P., and Berconsky, J.: The Cyanosis in Mitral Stenosis, Am. Heart J. **17**: 1, 1939.
38. Dexter, L.: Personal communication.
39. Riley, R. L., Himmelstein, A., Motley, H. L., Weiner, H. M., and Cournand, A.: Studies of the Pulmonary Circulation at Rest and During Exercise in Normal Individuals and in Patients with Chronic Pulmonary Disease, Am. J. Physiol. **152**: 372, 1948.
40. Stead, E. A. Jr., Warren, J. V., Merrill, A. J., and Brannon, E. S.: The Cardiac Output in Male Subjects as Measured by the Technique of Right Atrial Catheterization. Normal Values with Observations on the Effect of Anxiety and Tilting, J. Clin. Investigation **24**: 326, 1945.

PANCREATITIS IN METHYL ALCOHOL POISONING

IVAN L. BENNETT, JR., M.D., THOMAS C. NATION, M.D., AND
JAMES F. OLLEY, M.D.
ATLANTA, GA.

DURING a five-day period in October, 1951, more than 300 patients who had ingested bootleg whiskey containing 35 per cent methyl alcohol were treated at Grady Memorial Hospital. In general, they presented the classic symptoms of wood alcohol poisoning: visual disturbances from mild blurring to total blindness, severe abdominal pain, nausea, headache, and central nervous manifestations ranging from dizziness to convulsions and coma. The most obvious physiologic disturbance produced in human beings by methanol is severe acidosis, as evidenced by reduction in plasma carbon dioxide combining power. This was present in many of our patients. The mechanism of the acidosis is incompletely understood, although the breakdown of methanol in the body to formic acid and formaldehyde is thought to play some role in its production.

It is the purpose of the present report to describe the occurrence of pancreatitis after ingestion of methyl alcohol. Although post-mortem findings in methanol poisoning have been described extensively, we have found only two descriptions of pancreatic involvement. In 1930, Burhans[1] stated that hemorrhagic pancreatitis was found at autopsy in every one of 11 cases of methyl alcohol poisoning examined by him. Keeney and Mellinkoff[2] noted a few small areas of hemorrhage in the pancreas of one of six fatal cases of methyl alcohol intoxication.

MATERIAL FOR STUDY

Serial serum amylase determinations were performed on 14 patients selected at random from those hospitalized for methyl alcohol poisoning. All had symptoms of severe intoxication with acidosis, visual disturbances, etc. None received morphine or other narcotic which might influence serum amylase levels.[3, 4] All were given sodium bicarbonate in 3 or 5 per cent solution intravenously in amounts sufficient to restore the plasma carbon dioxide combining power to normal, and all but one of these patients survived. In nearly all instances, a period of twenty-four to seventy-two hours elapsed between ingestion of methanol and onset of symptoms, so that few amylase determinations were done during the first forty-eight hours after the poison was drunk. Amylase was determined by a modification of the Somogyi method.[5] In our laboratory, the normal limits for serum amylase are listed as 80 to 150 units, but figures of 50 to 200 units are sometimes seen in apparently normal subjects.

Material for histologic study consisted of tissues obtained at autopsy from 10 fatal cases during the outbreak of October, 1951. In addition, sections of pancreas from seven victims of methyl alcohol who died in December, 1946, were reviewed.

SERUM AMYLASE DETERMINATIONS

Ten of the 14 subjects tested had initial serum amylase levels exceeding 300 units. Elevated values were found as late as the eighth day after ingestion

From the Medical Service, Grady Memorial Hospital, and the Departments of Medicine and Pathology, Emory University School of Medicine, Atlanta, Ga.
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of methanol. In general, there was prompt return of amylase activity to normal levels with convalescence. One patient, whose amylase was 362 units three days after drinking methanol, died, and at autopsy extensive pancreatic necrosis was found. Another, whose serum was tested repeatedly, showed consistently normal amylase activity. Fig. 1 portrays the course of the serum amylase activity in 12 of the 14 patients. Table I compares initial amylase levels with plasma carbon dioxide combining power at the time of admission (in general, a measure of severity of methanol poisoning).

Table II shows the course of serum amylase in the only patient in whom persistent elevation was found. There was a slow increase in levels until the 42nd day, the time of last observation. He was asymptomatic and had no evidence of pancreatic or renal insufficiency. Unfortunately it was not possible to trace him for further observation.

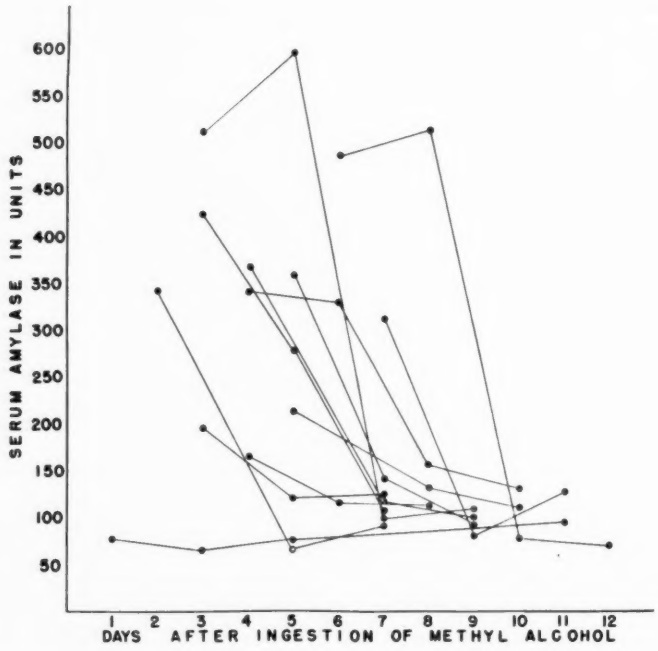

Fig. 1.—Course of serum amylase in twelve patients with methyl alcohol poisoning.

HISTOLOGIC FINDINGS

In 13 of the 17 patients pancreatitis was found. The lesions varied from scattered areas of acinar destruction with edema of the stroma to a striking hemorrhagic necrosis easily recognizable in the gross. Histologic study indicated that the earliest changes were degranulation and increased basophilism of the acinar cells with pooling of secretion in the ducts. This was followed by edema of the connective tissue of the lobules. The next step in the process appeared to be beginning necrosis of blood vessel walls, both arteries and veins,

TABLE I. TABLE SHOWING INITIAL PLASMA CARBON DIOXIDE COMBINING POWER, HIGHEST SERUM AMYLASE, AND RESULTS OF TREATMENT IN FOURTEEN PATIENTS WITH METHANOL POISONING

PATIENT	INITIAL CO_2 COMB. POWER (MEQ.)	HIGHEST AMYLASE RECORDED	DAY AFTER INGESTION	REMARKS
1	9.0	312 units	7th	Recovered
2	21.0	343	2nd	Recovered
3	7.7	356	5th	Recovered
4	4.8	166	4th	Recovered
5	13.0	598	5th	Recovered
6	13.0	216	6th	Recovered
7	6.5	424	3rd	Recovered
8	12.0	193	3rd	Recovered
9	12.0	342	4th	Recovered
10	n.d.	361	4th	Recovered
11	10.5	512	8th	Recovered
12	8.0	90	11th	Recovered
13	10.5	362	3rd	Died 4th day
14	15.0	1,111	42nd	Asymptomatic

resulting finally in extravasation of blood throughout the parenchyma with massive hemorrhagic destruction. The findings suggest that the greatest part of the damage is secondary to vascular injury and hemorrhage. In one of the four cases in which no frank pancreatitis was found there was definite edema of the stroma and occasional small foci of beginning necrosis were visible in the walls of the intralobular arterioles, although this had not progressed to the point of extravasation.

DISCUSSION

The finding of elevated serum amylase in 10 of 14 patients after ingestion of methanol and of pancreatic necrosis in 13 of 17 fatal cases justifies the conclusion that one of the features of methyl alcohol poisoning is pancreatic damage. The mechanism of this pancreatitis is not clear, but certain factors deserve consideration.

There appears to be a relation between ingestion of ethyl alcohol and acute pancreatitis. It has been estimated that 20 per cent of patients with acute pancreatitis are alcoholics. In a review of the literature, Ivy and Gibbs[6] found that 25 per cent of patients dying of acute alcoholism showed pancreatitis and that serum amylase was elevated in 24 per cent of alcoholic patients. Although the cause of pancreatitis associated with ethyl alcohol ingestion is unknown, several factors such as low protein diet, possible stimulation of secretion, etc., have been considered. Ivy and Gibbs were inclined to believe that

TABLE II. COURSE OF SERUM AMYLASE IN PATIENT 14 WHO WAS LOST TO FOLLOW-UP AFTER THIS TIME. HE WAS ASYMPTOMATIC WHEN LAST SEEN

DAYS AFTER INGESTION	SERUM AMYLASE
4	402 units
6	291
8	354
22	896
24	801
35	906
42	1,111

duodenitis with obstruction of pancreatic outflow is the most important etiologic factor. The bootleg liquor consumed by the patients in our series in 1951 contained less than 4 per cent ethanol and the mixture consumed by the seven patients who died in 1946 contained no ethyl alcohol at all. In only one of the autopsy protocols in our series was edema of the duodenal mucosa mentioned; in several, petechiae were noted, but in most, the mucosa was described as normal. The possible role of acidosis in the production of methanol pancreatitis should be mentioned. Pancreatitis is not ordinarily a feature of severe diabetic acidosis but Ivy and Gibbs[6] mention three instances of pancreatitis in children dying in diabetic acidosis. Furthermore, elevation of serum amylase is a common occurrence in uremia. Although reduction in renal clearance of amylase and the frequent finding of severe vascular disease in the pancreas in uremia can explain high serum amylase activity in renal insufficiency, it may well be that the concomitant acidosis in many uremia patients is also a factor.

Excruciating upper abdominal pain is a common finding in methanol poisoning. It may be accompanied by muscle rigidity of such extent that an acute surgical process is suspected. This pain has been explained on the basis of spasm of the smooth muscle of the gut and the finding of intestinal contractures has been emphasized at necropsy.[7] It seems likely that pancreatitis is responsible for much of the severe abdominal pain which is a prominent feature of methyl alcohol intoxication. In patients with acidosis due to methanol, the intravenous infusion of sodium bicarbonate characteristically produces striking symptomatic relief with restoration of consciousness, relief of visual disturbances (but apparently little effect on later permanent ocular damage) and alleviation of abdominal pain. This prompt response of pain to alkalinization could also be interpreted as evidence for the role of acidosis in the production of the pancreatic lesion. It is pertinent to point out that the importance of acidosis per se in the production of the manifestations of methyl alcohol poisoning is still a controversial issue.[7-9]

The possibility remains that the pancreatic lesions are the result of some direct action of methanol. The fact that there is a latent period after ingestion until onset of abdominal pain just as there is for the other manifestations of methanol poisoning such as acidosis and visual disturbance suggests that the breakdown products of methanol oxidation are the offenders.

SUMMARY AND CONCLUSIONS

Elevation of serum amylase was observed in 10 of 14 patients with methyl alcohol poisoning. At autopsy, pancreatic necrosis was present in 13 of 17 fatal cases. The pathogenesis of pancreatic injury after ingestion of methyl alcohol is not clear but histologic study suggests that vascular injury with subsequent hemorrhage is responsible for most of the observed changes. The role of acidosis in the production of pancreatitis in methyl alcohol poisoning remains to be determined. It is suggested that pancreatitis may be the cause of the severe abdominal pain which frequently accompanied methanol intoxication.

REFERENCES

1. Burhans, E. C.: Methyl Alcohol Poisoning (a clinical and Pathological Study of 11 Fatal Cases), Illinois M. J. **57**: 260-263, 1930.
2. Keeney, A. H., and Mellinkoff, S. M.: Methyl Alcohol Poisoning, Ann. Int. Med. **34**: 331-338, 1951.
3. Gross, J. B., Comfort, M. W., Mathieson, D. R., and Power, M. H.: Elevated Values for Serum Amylase and Lipase Following Administration of Opiates: A Preliminary Report, Proc. Staff Meet. Mayo Clin. **26**: 81-87, 1951.
4. Bennett, I. L., and Burgess, R. O.: Acute Morphine Poisoning with Manifestations of Pancreatitis, J. A. M. A. **148**: 938-939, 1952.
5. Somogyi, M.: Micro-methods for the Estimation of Diastase, J. Biol. Chem. **125**: 399, 1938.
6. Ivy, A. C., and Gibbs, G. E.: Pancreatitis: a Review, Surgery **31**: 614-642, 1952.
7. Røe, O.: Methanol Poisoning, Acta Med. Scandinav. **126**: Supplement 182, 1946.
8. Agner, K., Höök, O., and von Porst, B.: The Treatment of Methanol Poisoning with Ethanol With Report of Two Cases, Quart J. Stud. Alcohol, **9**: 515-522, 1948-49.
9. Røe, O.: The Roles of Alkaline Salts and Ethyl Alcohol in the Treatment of Methanol Poisoning, Quart. J. Stud. Alcohol **11**: 107-112, 1950.

BRONCHOSPIROMETRY

IV. Ambient-Air and Oxygen-Recording Bronchospirometry

Edward A. Gaensler, M.D., and Lt. David W. Cugell, Medical Corps,
Army of the United States
Boston, Mass.

INTRODUCTION

THE literature on split functional studies, the effects of resistance breathing, and the complications of, and contraindications to, the procedure have been reviewed in previous papers of this series.[1-3] The technique of bronchospirometry and the interpretation of the tracings obtained with twin spirometers, arranged to record on one drum, have been described in detail.[3]

Differential oxygen uptake, ventilation, and vital capacity are easily obtained with closed system spirometers. The Knipping type instrument was modified by Björkman[4] and the Benedict-Roth Spirometer was specially adapted by Gebauer[5] for use during bronchospirometry. We[6] have used the latter modification and have found this twin spirometer satisfactory during the course of 1,000 examinations.

The advantages of a direct recording system for respiratory analysis are apparent. Relative ventilation of the two lungs can be observed after the first breath, and relative oxygen uptake can be judged after the first few breaths. If the procedure is interrupted by slipping of the catheter, sudden leaks, bursting of a balloon, uncontrollable cough, or excessive secretions, a record of some value can nevertheless usually be salvaged. Further, by comparing the spirograms, the appearance of the chest roentgenogram and the position of the catheter, it is possible to identify occlusion of a bronchial orifice or an improperly functioning apparatus shortly after initiation of the test. Air leaks from one lung to the other or to the outside, and mechanical occlusion of one catheter lumen are recognized immediately. Finally, irregular or labored breathing is observed on the kymograph, and the cause can be investigated.[3]

This type of closed spirometry has four distinct disadvantages: (1) The inertia of the spirometer bells floating in water is considerable and causes some distortion of the breathing pattern. It may even cause faulty registration of volumes.[7] Because of the artificial conditions of resistance breathing during bronchospirometry,[2] and because the procedure is usually applied during quiet breathing, this factor is probably of little importance. (2) Calculation of minute ventilation is laborious because all the tidal volumes for several minutes must be measured and added. (3) The patients' lungs are subjected to constantly changing gas composition, since oxygen is removed from the spirometer and some nitrogen is added from the lungs. This is most objectionable when function is distributed unequally between two lungs, since after a while the major lung breathes gas of lower oxygen content than the poorly functioning lung which has removed less oxygen from its spirometer. (4) The initial oxygen concentration in the spirometers must be considerably higher than that of room air to avoid

From the Physiology Laboratory, Thoracic Surgery Service, Sanatorium Division of the Boston City Hospital, Mattapan, and the Department of Surgery, Boston University School of Medicine; and from the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

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anoxia later during the procedure. Because the data obtained from bronchospirometry are reported as relative percentages only, the high oxygen concentrations should not affect the results in normal subjects or patients with symmetrical pulmonary disease. In unilateral involvement the damaged lung may be able to take advantage of the oxygen rich mixture while the well lung cannot. This may cause not only an alteration in total ventilation and oxygen uptake, of no concern in bronchospirometry, but also a shift of ventilation and oxygen uptake from the normal to the diseased lung. It is therefore not certain that the relative performance of the two lungs, as recorded by closed circuit spirometry, is the same as that encountered during room air breathing.

All these disadvantages are obviated by open circuit spirometry.[8] Here the two external catheter orifices are attached to two inspiratory valves open to room air and to two expiratory valves connected to Douglas bags. After five to ten minutes the volumes of gas in the two bags are measured, and relative minute ventilation is obtained directly. For calculation of relative oxygen uptake and carbon dioxide output it is necessary to analyze the contents of the bags with a Haldane or other gas analyzer. Although this technique provides constant room air breathing it has not generally found favor in bronchospirometry because of the lack of a visual record of the respiratory effort. Leaks, malplacement, and slippage of the tube may go unnoticed, and relative vital capacities with their subdivisions cannot be obtained by the open circuit technique.

A combination of the open and closed circuit methods has been used by Wright.[9] After introduction of the catheter, vital capacities are obtained by the closed technique using large spirometers filled with room air. When correct placement and absence of leaks have been assured from a two-minute observation of the tracing, the airways are switched into an open circuit for collection of expired air; oxygen uptake, carbon dioxide output, and minute ventilation are obtained after gas analysis. A combination of these two methods has not proved satisfactory in our hands. Displacement of the catheter during the second part of the procedure due to cough or sudden motion occasionally went unnoticed until completion of gas analyses. Duplicate gas analyses of four samples obtained from each of three to five patients in one morning were tedious and time consuming.

The problem of obtaining a visual record during spirometry while supplying the patient with gas of an unchanging composition has intrigued investigators for many years. Interest was stimulated by Uhlenbruck's[10] observation that oxygen uptake could be increased temporarily in certain patients with cardiorespiratory disease by increasing the partial pressure of oxygen in the inspired gas.

We have been interested in ambient or room air breathing for recording of the respiratory level prior to functional residual capacity determination by the open circuit method and for use during bronchospirometry. We had the opportunity to observe an instrument designed by Maloney[11] for recording of respiratory excursions during closed system endotracheal anesthesia. This consisted of an airtight plastic bubble placed around an anesthesia bag. An opening in the bubble was connected to a spirometer whose valves and soda lime cannister had been removed. As the patient breathed out, air was displaced from the bubble by the increasing size of the anesthesia bag, and the spirometer bell rose. The reverse took place on inspiration. From this we have adapted a system for ambient air breathing in which the inspired air remains completely unaltered, which does not require intermittent or continuous addition of oxygen and which incorporates all the advantages of continuous recording of respiratory excursions. It consists of an airtight metal drum with a plastic window. A balloon is sealed within this drum with a large rubber stopper. The balloon is filled with room air, and the patient inspires room air from the balloon through a respiratory valve and expires into the box around the balloon through another valve. A spirometer is connected to the drum, and the rise and fall of its bell reflects the volume changes within the patient's chest, although the flow of respired air is not directed through the spirometer. The balloon supplies enough air for quiet ventilation for four to seven minutes, and has been used in this fashion for determination of the respiratory level prior to residual air determination.[12-14]

Since then, a number of other publications have come to our attention reporting the use of this system for separating inspired from expired air. Roth[15] was the first who designed

such an apparatus for determination of the basal metabolic rate during room air breathing. About the same time it was shown that the metabolic rate is not ordinarily affected by the oxygen tension of the inspired air, and his apparatus was never constructed. Sonne[16] was the first who actually utilized the bag-within-box principle for separation of inspired from expired gas during spirometry. The motion of the spirometer served to activate a series of alveolar gas samplers, and the distribution of the tidal volume within the lungs was studied by introduction of hydrogen from a second bag within the box. This technique was later amplified by Sonne and Georg.[17, 18] Donald and Christie[19] used boxes of 100 liter capacity with 80 liter Douglas bags. Two such boxes, which could be used alternatingly, were incorporated within a single portable instrument. They were able to observe changes in oxygen uptake, ventilation and respiratory quotient with use of gases of different composition. Georg[20] modified the Sonne design. He used a wooden box of about 750 liter capacity, sealed with water traps, in which two 150 liter Douglas bags were suspended. Tracings were obtained by means of a Krogh spirometer connected to the box. The bags supplied enough air for ten minutes of exercise.

The problem of recording spirometry with constant gaseous composition has been approached from two other directions: either the capacity of the system has been increased, or oxygen has been added to the system to compensate for the patients' metabolic requirement. The volume of the closed system has been augmented by increasing the size of the spirometer[21, 22] or by interposing a large airtight box in the inspiratory circuit.[23] By these methods resting spirometry could be carried on for several minutes without greatly altering the composition of the inspired gas. However, they did not solve the problem entirely, for the composition of the gas remained only approximately constant. Also, if the box or spirometer was large enough to accomplish sufficient dilution, then small temperature changes affected the volume of the container greatly, and the slope of oxygen uptake line was considerably falsified. Thus, if a box of 200 liters was used, as was suggested by Arnaud and associates,[23] a rise of temperature within the box from 20° C. to 21° C. increased the volume within the system by 683 ml., or more than the entire oxygen uptake for approximately three minutes.

The second approach to the problem has been the addition of oxygen to the system to compensate for oxygen removed by the patient. This has been done intermittently or continuously. With the intermittent method, the spirometer was filled with room air and enough oxygen was added every one or two minutes to return the spirometer to its original volume.[10, 21, 22, 24] This method, too, provided for only approximately constant gas composition. The more frequently refills were made, the more constant was the gaseous composition. However, the refills resulted in frequent interruptions of the slope, and oxygen uptake could no longer be read with accuracy. To circumvent this frequent interruption of the ventilation slope, Nager[25] described a system consisting of two spirometers. The respiratory circuit could be switched from one spirometer to the other by a single maneuver while the recording pen was moved by an electromagnet to the spirometer in use. Simultaneously, oxygen was added to the spirometer not in use. If switching was done frequently, the oxygen tension could be maintained approximately constant without disturbing the continuity of the ventilation slope.

The continuous methods have been used less often because of the intricate techniques required for adding oxygen continuously at the patients' rate of removal. The simplest way to accomplish this was to add just enough oxygen by manual control of a valve to keep the slope of the respiratory excursions on a horizontal line. Oxygen was added from a second spirometer by screw clamp control,[26, 27] or from a tank by regulating the flow with a reduction valve and a finely graduated flow meter.[28, 29] These techniques required constant attention by an experienced observer and did not permit direct reading of oxygen uptake from the respiratory tracing.

A number of methods have been described for automatic continuous addition of oxygen. The "dosimeter" by Bjerknes and Scholander[30] consisted of two superimposed containers, the upper of which was filled with water and the lower with oxygen. Water slowly flowed from the upper into the lower container. Oxygen was thereby displaced from the lower container into the spirometer and, at the same time, air was sucked from the spirometer into the

upper water container. The fall of the water level was synchronized with the rise of the recording pen of the Krogh spirometer. The ventilation curve continued to have an upward slope and could be used to measure oxygen uptake while the oxygen tension of the inspired gas remained unchanged. Eckman and Barach[31] used a system where the oxygen addition was controlled by the inspiratory position or the lowest spirometer volume. This had the disadvantage that a sigh or a deep breath caused a sudden injection of oxygen, thereby raising the spirometer oxygen tension for some time. The injection of oxygen was controlled by the expiratory position or largest spirometer volume in a system described by Anthony and Rohland.[32] The oxygen supply was regulated by an electromagnetic valve which was intermittently opened by the counterweight of the spirometer bell dipping into a pool of mercury as the amount of gas in the spirometer decreased. The most complex electro-mechanical oxygen replenisher was designed by Rossier and Wiesinger.[33] The instrument incorporated a device which continuously "palpated" the respiratory level in the expiratory position. Oxygen was added upon the slightest variation in this level and could be added fast enough to permit use of the instrument during exercise.

These continuous techniques are imperfect in many ways. In spite of automatic maintenance of a constant spirometer volume, the oxygen tension decreases slowly, first, because the added tank oxygen contains 0.5 to 2 per cent inert gas and second, because the wash bottle or soda lime container does not remove all the exhaled carbon dioxide completely, which is then added to the volume in the spirometer. The use of most automatic supply systems does not permit direct reading of oxygen uptake from the tracing without accessory spirometers or flow integrators. Finally, any spontaneous variations in the functional residual capacity (pulmonary resting position) are interpreted by the regulators as variations in oxygen uptake and are automatically and erroneously compensated. The functional residual capacity may vary by several hundred cubic centimeters from time to time in the same patient at rest.[34, 35] Such variations occur regularly in patients with obstructive ventilatory insufficiency. The oxygen tension within the spirometer therefore changes in spite of constant automatic maintenance of a horizontal respiratory base line when the regulator misinterprets a rise in the functional residual capacity as a sudden increase in oxygen uptake.

All of these devices were primarily designed for the exploration of the "oxygen deficit," anoxia experiments, and the study of metabolism during exercise. Only Tulou[36] applied one of these methods to bronchospirometry by interposing "Arnaud boxes"[23] in the inspiratory circuits of a twin spirometer to approximate room air breathing. Others, recognizing the unphysiologic effect of oxygen breathing during bronchospirometry, have turned to the open circuit method.[8, 9] Wright and Woodruff[9] have compared the relative oxygen uptake of diseased lungs rebreathing air from a spirometer and breathing room air in an open circuit. In forty-one patients examined in this manner, the difference of participation in oxygen uptake between the two lungs was more than 5 per cent in half the cases, and as much as 15 per cent in some. However, closed spirometry was performed for only two minutes, and the authors felt that this was often too short a time for accurate determination of the respiratory slope. Björkman and Carlens[37] were the only investigators who reported bronchospirometry using both room air and oxygen in the same patients. The method used to accomplish this was not stated. They found that the share of oxygen uptake of the inferior lung decreased from 2 to 5 per cent in sixteen patients on transition from oxygen to air. Unfortunately, they seemed to have always used oxygen before room air so that the transition from one type of gas to the other must have been gradual, as oxygen was rinsed from the lungs and tissues during the later air breathing period.

MATERIALS AND METHODS

The Box-Balloon System.—All present drum-balloon combinations used by the authors consist of cylindrical airtight drums of 30-liter capacity with large plastic windows. They are fitted with 30-liter meteorological balloons which are inexpensive and are made of very thin neoprene. This material transmits pressure changes without lag, and is much less permeable to carbon dioxide than natural rubber. Initially, 100 liter Douglas bags were used

with correspondingly larger drums. The relatively stiff bags caused considerable damping of the respiratory excursions and, with increasing drum volume, the determination of oxygen uptake became less accurate. Small temperature changes caused large changes in volume, a fact previously discussed. Georg,[20] with his large wooden box, had to use a copper baffle in the expiratory circuit to equilibrate the expired air with room temperature. Again, because only relative values are reported from bronchospirometry, these volume changes are relatively unimportant if both lungs consume the same amount of oxygen, and temperature changes are equal in both drums. The increase in volume due to temperature rise leads to erroneous results if one lung is nearly functionless. The 30-liter balloon is suitable for seven to fifteen minutes' ventilation during bronchospirometry, depending on whether resting ventilation is equally distributed between the two lungs or is carried on chiefly by one lung. During this time, the temperature, measured by a thermometer built into the drum, rarely changed more than 0.2° C. During ten minutes' spirometry, this maximal temperature variation caused a change in volume of only 2.5 ml. per minute.

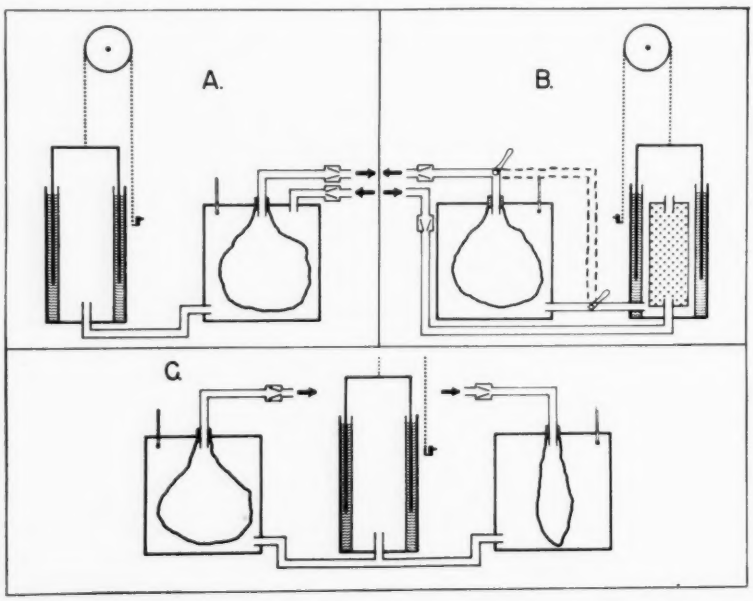

Fig. 1.—Three drum-balloon systems for ambient air recording spirometry. *A*, Air is inhaled from the balloon and returned into the drum around the balloon. The spirometer merely records volume changes. This apparatus produces a spirogram with downward slope if the respiratory quotient is less than unity. Introduction of a soda lime cannister in the expiratory circuit produces a conventional spirogram whose upward slope corresponds to oxygen uptake. *B*, The patient exhales through the soda lime cannister of the spirometer into the box. Two three-way valves placed at the junction of the tubing drawn in solid and in broken lines permit instantaneous switching from ambient air to conventional closed spirometry. *C*, The inspiratory balloon on the left is filled with room air and the balloon on the right side is emptied. The balloon on the right slowly fills during the test period and samples can later be removed for expired air analysis.

The three box-balloon systems, used by us, are illustrated in Fig. 1. In the first and simplest system (Fig. 1, *A*) the patient inhales room air from the balloon through a one-way valve facing the patient and exhales into the box around the balloon through a one-way valve facing the box. A conventional spirometer, with valves and soda lime cannister removed, is connected to the box with large rubber tubing. During inspiration, the volume in the box is reduced as the balloon decreases in size and the spirometer bell descends. When the patient exhales into the box around the balloon the volume increases and the spirometer bell rises.

In the system illustrated the respiratory base line falls slightly, since the respiratory quotient is ordinarily less than 1.0. If a soda lime cannister is introduced in the expiratory circuit the slope of the base line rises and corresponds to oxygen uptake.

The second system (Fig. 1, *B*) is similar to the first except that balloon-box ambient air spirometry and conventional oxygen spirometry can be interchanged instantaneously and without interrupting the respiratory base line. This is made possible by the introduction of two three-way valves at the point where the solid and the broken lines meet. This system is used in the bronchospirometer described below.

The third system (Fig. 1, *C*) makes it possible to obtain a spirogram, and at the same time provides for later analysis of the expired air. The inspiratory balloon, on the left, is initially filled with room air and the balloon on the right is completely empty. During spirometry, the balloon on the left gradually empties and the one on the right fills with expired

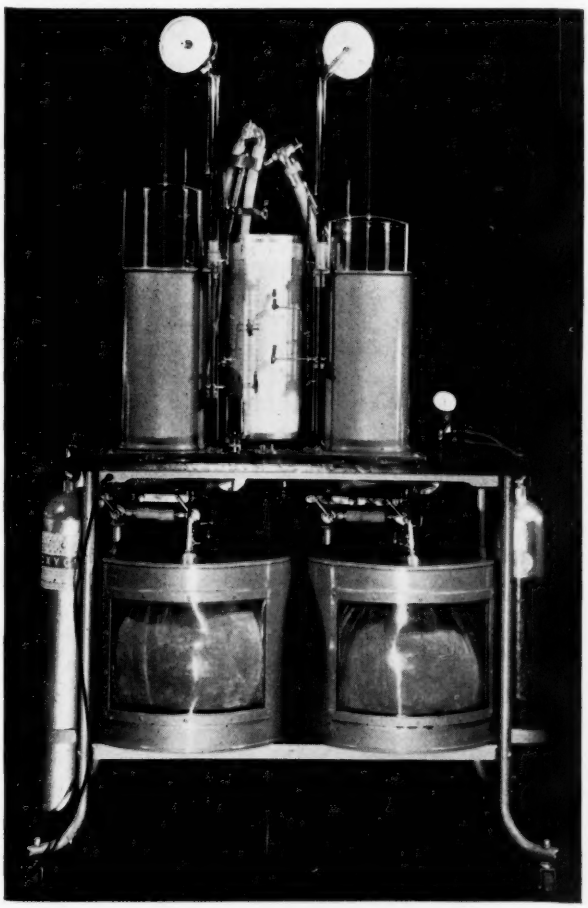

Fig. 2.—The ambient air bronchospirometer incorporates box-balloon system *(B)* of Fig. 1. Above the table level are the two spirometers with thermometers and ventilograph attachments, a common recording drum with four pens for recording respiratory excursions and integrated ventilation of each lung, a speed selector, a light for observation during fluoroscopy and an aneroid manometer for inflation of the cuffs of the catheter. Two tanks are provided, one with oxygen for filling of spirometers and one with compressed air for filling of the balloons. Below the table level are two airtight drums with plastic windows and thermometers; balloons are suspended within. Above each drum are two three-way valves coupled in tandem to permit instantaneous change from room air to oxygen-enriched atmosphere. Below the table level, and facing the patient, are four low resistance respiratory valves housed in translucent plastic.

gas. The spirometer records a conventional spirogram by reflecting volume changes in both boxes. Samples of expired air can later be taken for analysis from the balloon on the right. This arrangement is useful for study of alveolar-arterial oxygen difference, for determination of cardiac output during venous catheterization, and for any other experiment where a conventional spirogram as well as ana'ysis of expired gas is desired.

*The Ambient Air Bronchospirometer**.—The bronchospirometer shown in Fig. 2 combines two box-balloon systems of the type shown in Fig. 1, *B* with two 9-liter Benedict-Roth spirometers. In addition to combining the advantages of recording spirometry with those of constant composition room-air spirometry, the spirometers are fitted with Reichert ventilographs,[38] which make possible direct reading of differential minute ventilation. Above the table level, four recording pens are seen in front of the common recording drum. The lower two pens record tidal excursions, vital capacity, and oxygen uptake. The upper two pens are connected to the ventilographs. Other controls above the table level include a one-inch and five-inch per minute speed selector, a small lamp for observation of the tracing during fluoroscopy, and an aneroid manometer for control of inflation of the catheter balloons. Both spirometers are fitted with thermometers and soda lime cannisters. Mounted under the table are the two 30-liter airtight drums fitted with balloons. Large windows of heavy transparent plastic material comprise the posterior aspect of the cans. They enable observation of the extent of inflation of the balloons. Both cans are fitted with thermometers and small pet cocks. Two outlets of large bore are mounted above each drum. One outlet opens into the drum and the other into the balloon. The two outlets are guarded by large-bore, three-way gas cocks connected in tandem by a transverse handle. With the handles in the position illustrated in Fig. 2 respiration proceeds as indicated by the solid lines of Fig. 1, *B*. The patient breathes room air from the bag and exhales through the soda lime cannister of the spirometer back into the outside of the box around the balloon. Movement of both handles to the right connects the patient to the spirometer as indicated by the broken lines of Fig. 1, *B*, permitting conventional oxygen breathing. The patient inhales from the spirometer and exhales back into the spirometer through the soda lime cannister. The balloon outlet is also fitted with a pet cock. To fill the balloons with room air, the handles are turned to the right to isolate the drums from the spirometers, and both the drum and balloon pet cocks are opened. Compressed air from the tank on the right can then be introduced into the balloon opening or suction can be applied to the box opening. The spirometers are filled with oxygen from the tank on the left through two additional pet cocks. The respiratory valves are mounted under, and just in front of the table and connect directly with the tubing to the catheter. The valves are of the low resistance "J" type and are housed in transparent plastic to facilitate observation of their action. The valves and cans are easily removed for replacement and inspection. The balloons are likewise easily removed, but one pair of balloons has been in daily use at the author's laboratories for over three years without becoming defective. The entire instrument is mounted on casters and is semi-portable.

RESULTS

Results of ambient air and oxygen bronchospirometry of three normal volunteers are shown in Table I. These volunteers and the patients were chosen from a larger group because they all had studies with room air first, followed immediately by conventional high oxygen tension spirometry. The tracings were of sufficient duration, quality, and regularity, both with oxygen and air breathing, to make strict comparison possible.

In normal volunteers, respiration of the right lung, on the average, accounted for 57 per cent of the total oxygen uptake. The actual share of function of the right lung was somewhat higher than 53 per cent, generally accepted as normal in the literature. However, it agreed with a number of other studies of

*This instrument was made by Warren E. Collins, Inc., 555 Huntington Avenue, Boston, Mass.

TABLE I. RELATIVE OXYGEN UPTAKE DURING BRONCHOSPIROMETRY WITH ROOM AIR AND WITH OXYGEN IN NORMAL VOLUNTEERS

PATIENT	DISEASE	OXYGEN UPTAKE PER CENT OF TOTAL				MINUTE VENTILATION PER CENT OF TOTAL				VENTILATION EQUIVALENT, LITERS/100 C.C. OF OXYGEN			
		ROOM AIR		OXYGEN		ROOM AIR		OXYGEN		ROOM AIR		OXYGEN	
		RIGHT SIDE	LEFT SIDE	RIGHT SIDE	LEFT SIDE	RIGHT SIDE	LEFT SIDE	RIGHT SIDE	LEFT SIDE	RIGHT SIDE	LEFT SIDE	RIGHT SIDE	LEFT SIDE
1.	Normal volunteer	60	40	60	40	56	44	56	44	2.1	2.5	1.9	2.3
2.	Normal volunteer	59	41	59	41	60	40	60	40	2.4	2.5	2.1	2.2
3.	Normal volunteer	53	47	53	47	53	47	53	47	2.6	2.6	2.4	2.4
Mean		57	43	57	43	56	44	56	44	2.4	2.5	2.1	2.3

normal volunteers and patients with minimal tuberculosis performed at our laboratories. The extent of participation was the same during both oxygen and ambient air breathing. Mean ventilation for the right lung was 56 per cent of the total, corresponding well with oxygen uptake.

The ventilation equivalent, expressed as number of liters of ventilation for 100 ml. of oxygen absorbed, was about the same for both lungs. However, it was slightly lower for both lungs on oxygen than on room air breathing. A lower ventilation equivalent on oxygen breathing was observed throughout the study both in normal volunteers and patients with all degrees of pulmonary involvement. The change from room air to oxygen breathing was not considered responsible for the lowering of the ventilation equivalent in the normal volunteers, and only partly responsible in patients with pulmonary disease. In all these experiments room air bronchospirometry was carried out prior to oxygen spirometry. If the procedure had been reversed, a high oxygen concentration would have persisted in the patients' lungs and invalidated the room air results. From observation of a number of routine bronchospirometries carried on in duplicate with oxygen breathing, it was evident that considerable hyperventilation always occurred during the first period of observation. It was due to the unaccustomed resistance of the catheter and spirometer, and the patients' inability to feel the movement of air in the upper respiratory passages. After a few minutes of quiet breathing and a vital capacity effort, patients usually became accustomed to the catheter and voluntary hyperventilation was less marked during the second effort, independently of the oxygen tension used.

In twelve patients, moderate unilateral functional impairment was due to hemothorax, bronchogenic carcinoma, tuberculosis with or without thoracoplasty, lobectomy, and phrenic nerve interruption (Table II). The mean oxygen uptake of the involved lung was 34 per cent of the total during both room air and oxygen spirometry. Oxygen uptake was identical under both conditions in six of the twelve patients, and varied by no more than 2 per cent in the others. Ventilation was reduced to a lesser extent than oxygen uptake—a classical finding of bronchospirometry in unilateral disease. This caused a larger ventilation equivalent for the diseased lung than for the good lung. During both oxygen and room air bronchospirometry the involved lung ventilated nearly twice as much as the good lung for the same oxygen uptake. As in normal volunteers, both ventilation equivalents were lower during oxygen breathing.

Patient No. 14 (Table II) was selected from this group as a typical example. He was a 43-year-old man with moderately advanced left apical tuberculosis with cavitation (Fig. 3). The bronchospirogram had the same appearance during both room air and oxygen breathing (Fig. 4).

The last seven patients shown in Table III had almost functionless lungs on one side. The causes were bronchogenic carcinoma with distal atelectasis, far-advanced tuberculosis usually associated with atelectasis or empyema, traumatic hemofibrothorax, and, in one instance, congenital absence of the right pulmonary artery associated with a normal chest roentgenogram. During room air breathing the mean oxygen uptake of the diseased lung was only 3 per cent of the total. On oxygen breathing it was increased to 12 per cent. This fourfold in-

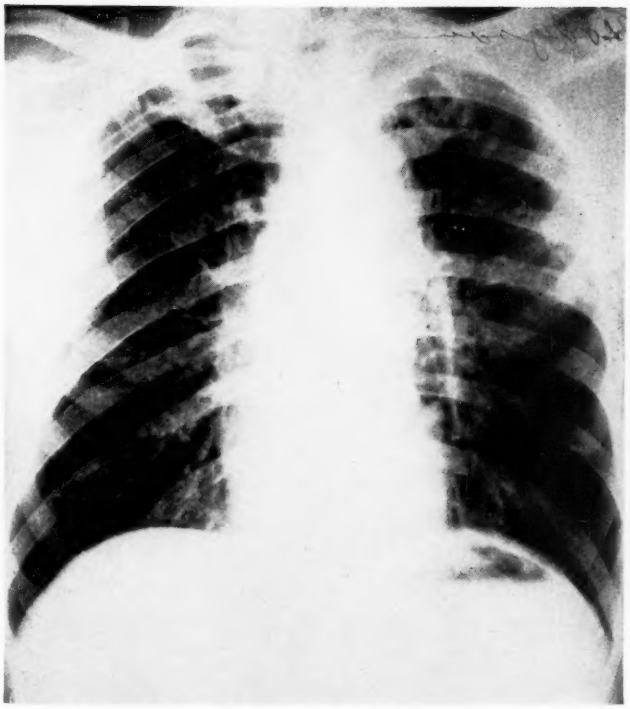

Fig. 3.—Chest roentgenogram of patient No. 14. Moderately advanced tuberculosis with cavities at the apex of the left lung.

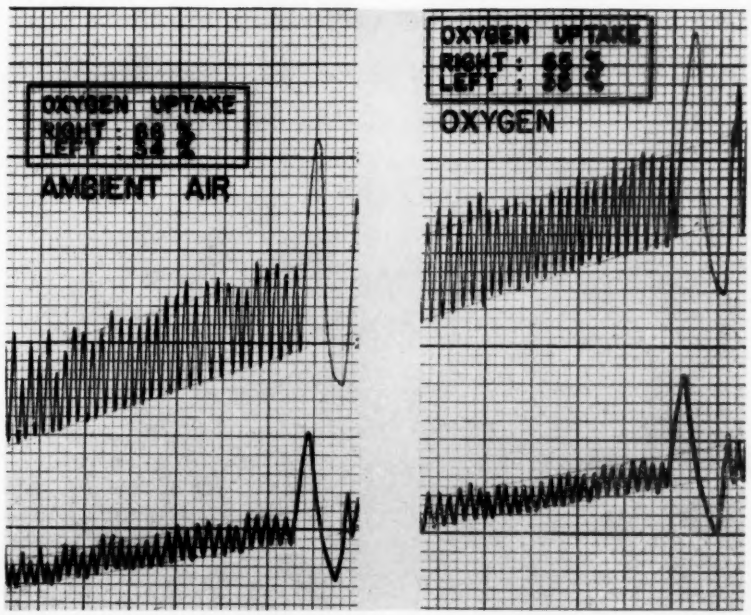

Fig. 4.—Bronchospirograms of patient No. 14. On switching from room air to oxygen the functional participation of the involved lung remained unaltered. In this and the following bronchospirograms the upper tracing comes from the right lung.

TABLE II. RELATIVE OXYGEN UPTAKE DURING BRONCHOSPIROMETRY WITH ROOM AIR AND WITH OXYGEN IN PATIENTS WITH MODERATE UNILATERAL DISEASE

PATIENT NO.	DISEASE	SIDE	OXYGEN UPTAKE, PER CENT OF TOTAL				MINUTE VENTILATION, PER CENT OF TOTAL				VENTILATION EQUIVALENT, LITERS/100 C.C. OF OXYGEN			
			ROOM AIR		OXYGEN		ROOM AIR		OXYGEN		ROOM AIR		OXYGEN	
			INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE
4.	Hemothorax, 50 per cent collapse	R	31	69	31	69	48	52	48	52	5.0	2.4	4.6	2.2
5.	Moderately advanced tuberculosis	L	33	67	35	65	38	62	40	60	2.3	1.9	2.5	2.0
6.	Moderately advanced tuberculosis	R	37	63	36	64	51	49	51	49	3.9	2.2	4.5	2.4
7.	Tuberculosis, phrenic paralysis	R	43	57	45	55	53	47	54	46	3.2	2.1	2.8	1.9
8.	Tuberculosis arrested, thoracoplasty	R	36	64	36	64	40	60	40	60	2.6	2.2	2.5	2.1
9.	Inactive tuberculosis, lobectomy	R	37	63	35	65	45	55	42	58	4.1	3.0	2.5	1.9
10.	Inactive tuberculosis, thoracoplasty	R	21	79	21	79	41	59	40	60	5.7	2.6	3.8	1.5
11.	Inactive tuberculosis, thoracoplasty	R	42	58	43	57	53	47	57	43	3.3	2.1	3.8	1.8
12.	Far-advanced tuberculosis	R	18	82	18	82	58	42	59	41	11.1	1.8	8.8	1.3
13.	Mod. advanced tuberculosis	R	39	61	39	61	44	56	44	56	2.5	2.1	2.4	2.0
14.	Mod. advanced tuberculosis	L	34	66	35	65	24	76	26	74	1.8	2.8	1.8	2.8
15.	Bronchogenic carcinoma, lower lobe	L	38	62	38	62	40	60	40	60	2.4	2.2	2.4	2.2
Mean			34	66	34	66	43	57	45	55	4.0	2.3	3.5	2.0

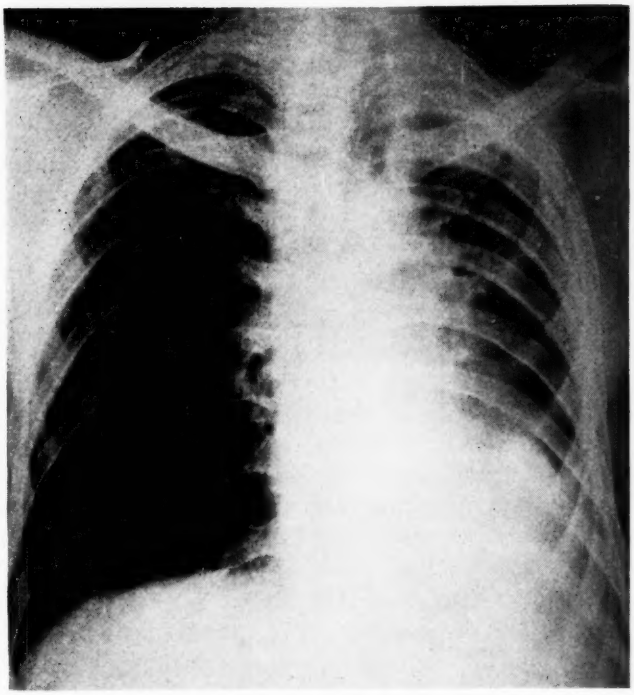

Fig. 5.—Chest roentgenogram of patient No. 16. Far-advanced tuberculosis, complicated by tuberculous empyema on the left.

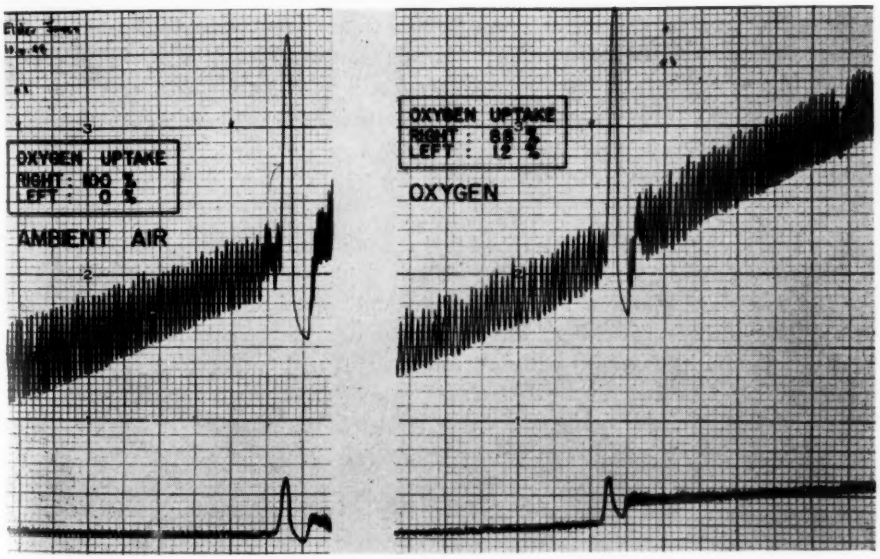

Fig. 6.—Bronchospirograms of patient No. 16. On room air breathing the left lung did not participate in oxygen uptake while on breathing an oxygen-enriched atmosphere the same lung was responsible for 12 per cent of the total oxygen uptake.

crease was attained by raising the oxygen tension in the inspired air from about 160 mm. to about 300 to 500 mm. of mercury. The shift in oxygen uptake of the seven individual patients was never less than 2 per cent, and as much as 12 per cent in two instances. Relative ventilation was not affected by the shift to oxygen spirometry. Again, as in the previous group, oxygen uptake was more severely impaired than ventilation, though the group was not as consistent in this respect. In two patients, one with hemofibrothorax and one with an absent pulmonary artery, the affected lung ventilated more than the good lung, a not unusual finding.[39]

Two patients of this last group were selected for individual presentation. Patient No. 16 (Table III) was a 40-year-old man with predominantly left-sided far-advanced pulmonary tuberculosis and a residual empyema due to an old, unsuccessful therapeutic pneumothorax (Fig. 5). The bronchospirogram (Fig. 6) had a different appearance on room air and oxygen breathing, though procedural hyperventilation was obviously more marked during the first effort. The left lung, represented at the bottom of the tracing, ventilated slightly, but the base line showed no elevation during room air breathing. With oxygen breathing there was a small but definite rise of the base line; the oxygen uptake was then 12 per cent of the total on the left.

The last patient, No. 22 (Table III), had an extensive thoracoplasty on the right six years prior to examination. The thoracoplasty failed, but further surgery was not attempted because of severe exertional dyspnea due to complicating emphysema. Pneumonectomy was feasible only in the complete absence of function on the right side. However, on the roentgenogram the right lung appeared partially aerated (Fig. 7) making bronchospirometry necessary. During oxygen breathing it was responsible for 8 per cent of the total oxygen uptake. On the basis of these studies, the operation was not done because it was felt that removal of even this little functioning lung tissue would not be tolerated. A few months later bronchospirometry was repeated with ambient air followed by oxygen (Fig. 8). Under normal conditions, breathing room air, the right lung did not participate in respiration. The lung was therefore removed, and after a stormy postoperative period the patient recovered and has been well for two years.

Patient No. 17 presented a physiologic problem entirely unlike the remaining patients. Although the roentgenographic appearance of the lung fields was essentially normal, angiocardiography did not reveal any pulmonary arterial blood supply to the right lung. There were no other apparent congenital cardiovascular defects. Bronchospirometry was performed under general anesthesia. Because of this, arterial hypoxia existed during the test, while in most other patients arterial oxygen saturation was presumably normal. Information obtained from bronchospirometry and blood gas analysis while room air and oxygen-enriched atmospheres were supplied permitted calculation of systemic arterial flow through the right lung. A detailed analysis of these findings will be made the subject of a separate report.[40]

Accuracy of the Method.—The increase in the share of oxygen uptake of the diseased lung, upon changing from room air to an oxygen-enriched atmosphere,

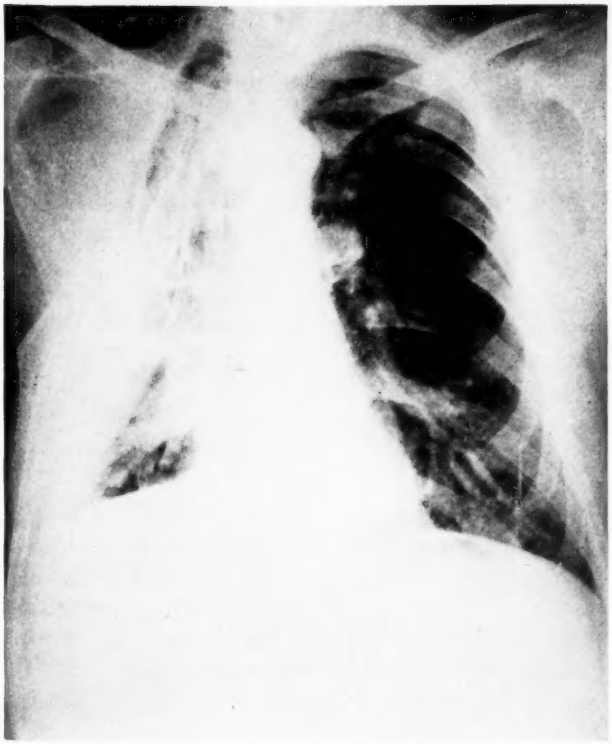

Fig. 7.—Chest roentgenogram of patient No. 22. Far-advanced tuberculosis and a ten-rib thoracoplasty on the right. The disease is complicated by pulmonary emphysema and fibrosis.

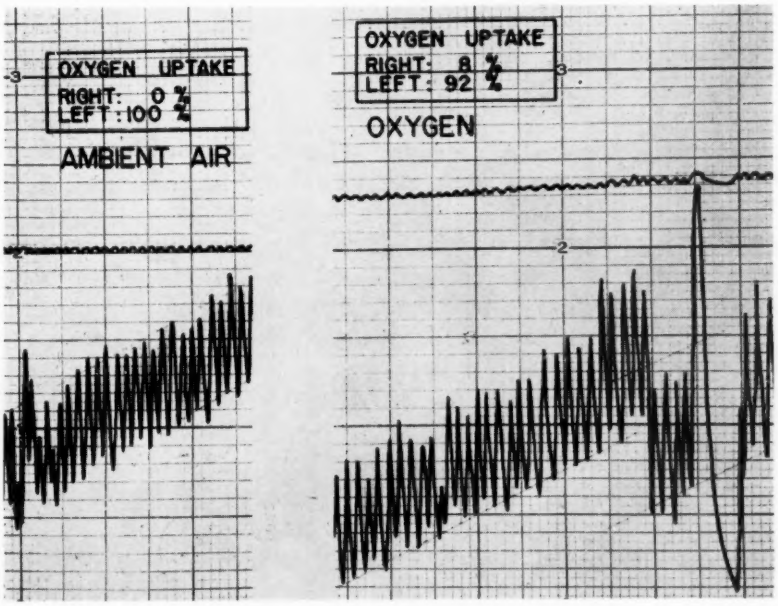

Fig. 8.—Bronchospirograms of patient No. 22. Although there was no participation in oxygen uptake on room air breathing the right lung took up 8 per cent of the total oxygen during oxygen breathing.

TABLE III. RELATIVE OXYGEN UPTAKE DURING BRONCHOSPIROMETRY WITH ROOM AIR AND WITH OXYGEN IN PATIENTS WITH "DESTROYED LUNG"

PATIENT NO.	DISEASE	SIDE	OXYGEN UPTAKE (PER CENT OF TOTAL)				MINUTE VENTILATION (PER CENT OF TOTAL)				VENTILATION EQUIVALENT (LITERS/100 C.C. OF OXYGEN)			
			ROOM AIR		OXYGEN		ROOM AIR		OXYGEN		ROOM AIR		OXYGEN	
			INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE	INVOLVED SIDE	UNINVOLVED SIDE
16.	Fibrocaseous tuberculosis, empyema	L	0	100	12	88	10	90	11	89	--	2.8	2.1	2.5
17.	Congenital absence, right pulmonary artery	R	0	100	18	82	55	45	55	45	--	1.4	6.7	1.2
18.	Far-advanced tuberculosis, empyema	R	4	96	10	90	5	95	5	95	2.2	2.2	0.9	2.4
19.	Far-advanced tuberculosis, atelectasis	L	6	94	8	92	14	86	14	86	5.2	2.2	2.6	2.2
20.	Bronchogenic carcinoma and atelectasis	L	2	98	10	90	12	88	12	88	16.0	2.5	3.3	2.5
21.	Extensive hemofibrothorax	L	9	91	19	81	53	47	55	45	16.0	1.4	7.6	1.4
22.	Far-advanced tuberculosis, 10-rib thoracoplasty	R	0	100	8	92	5	95	4	96	--	2.5	1.1	2.1
Mean			3	97	12	88	22	78	22	78	--	2.1	3.5	2.0

was often small. The limit of error of the technique should therefore be established. Conventional closed circuit oxygen spirometry is carried out in duplicate at our laboratories unless the procedure is interrupted by intolerable dyspnea, excessive accumulation of secretions, or uncontrollable cough.[3] Two successive four-minute periods, interrupted by a vital capacity effort, are deemed sufficient if breathing is regular and a good base line is obtained. If breathing is very irregular, periods from six to ten minutes are often required for satisfactory analysis. The position of the catheter is checked fluoroscopically and spot films of the trachea and upper bronchi are taken before, between, and after these two recording periods. The position of the catheter is often changed between the two tests. The first 320 duplicate tracings obtained in this manner were analyzed for the difference of results expressed in terms of per cent of total function. Results were then correlated with the three spot films. On 96 occasions the two tracings were not comparable due to accumulation of secretions, cough, technical difficulties or because the orifice of the left upper lobe was wholly or partially blocked by the distal balloon of the catheter during the first or second tracing.[3] Occasionally, either one or the other tracing was too short for satisfactory appraisal. Of the 224 duplicate tracings which were comparable, the participation of oxygen uptake of the involved lung expressed as per cent of the total oxygen uptake differed in the two tracings by 5 per cent or more on three occasions. The difference was 4 per cent three times, 3 per cent nineteen times, and 2 per cent or less in the remaining 199 duplicate tracings. The duplication of relative ventilation was not quite as good, and hyperventilation during the first effort appeared to favor the healthy lung. Consecutive vital capacity efforts duplicated poorest, incomplete efforts favoring the diseased lung.[3]

Since the oxygen uptake differed by only 2 per cent in 89 per cent of the duplicate tracings any larger difference during the present experiments was presumably due to the shift from room air to an oxygen-enriched atmosphere.

DISCUSSION

The amount of oxygen taken up by each lung depends on the partial pressure of oxygen within the alveoli, the permeability of the alveolar membranes, the oxygen tension of the pulmonary arterial blood, the time available for equilibration and the fraction of the right ventricular output delivered to each lung. Any one or all of these factors may be altered by oxygen breathing of both lungs. Of interest here is the mechanism by which the participation in oxygen uptake of the diseased lung may be increased out of proportion to the good lung.

The partial pressure of oxygen within the alveoli of the uninvolved lung is increased from about 100 mm. Hg to about 300 to 500 mm. by breathing of an oxygen-enriched atmosphere. A much higher oxygen tension cannot be obtained by closed system spirometer rebreathing because a considerable amount of nitrogen, present in the lungs and tubing at the time of room air breathing and a smaller amount liberated from the tissues during the oxygen breathing period, are added to the initially pure oxygen. The oxygen dissociation curve is in its flat portion when the alveolar oxygen tension is only 100 mm. Hg. The increase

of alveolar oxygen tension due to oxygen breathing of the well lung has no appreciable effect on its oxygen uptake since the blood leaving it is 96 per cent saturated during the ambient air breathing.[41] The situation may be very much the same in the diseased lung if the good portions of that lung are normally perfused, normally ventilated, and have a normal alveolar membrane. Its relatively low oxygen uptake must then be due to previous resection of the diseased portions or due to complete mechanical obliteration of the pulmonary arterial supply to the damaged areas by thrombosis, collapse, or kinking. More commonly there is a reduced pulmonary arterial blood flow to overdistended emphysematous areas and regions with distal bronchiolar disease. These segments are poorly ventilated because the local dead space is increased, the lumen of the supplying airway is diminished, and parenchymal and pleural elasticity are reduced. The partial pressure of oxygen in these regions is below normal during room air breathing and may fall to the level of that in the pulmonary arterial blood. High concentrations of oxygen in the inspired gas may raise oxygen tension in these underventilated areas sufficiently to effect capillary diffusion. This increases the relative participation of oxygen uptake of the diseased lung. Björkman and Carlens[37] have shown data from two patients of this type, one with a large cyst and the other with bronchiectasis. It is likely that the increased partial pressure of oxygen in diseased areas and in parts of the lung adjacent or distal to diseased areas plays a major role in increasing the relative oxygen uptake on that side when the inspired gas is enriched with oxygen.

The permeability of the alveolar membrane in the normal lung is sufficiently great so that, on ambient air breathing, practically no difference exists between the oxygen tension in the alveoli and in the capillaries.[42, 43] Again, the membranes of ventilated and perfused areas in the poorly functioning lung may be perfectly normal. More often there will be localized areas of edema, thickening, and fibrosis. Even if the damaged areas are relatively well ventilated, the diffusion coefficient for oxygen will be so high that little or no gas crosses the membrane. If, on oxygen breathing, the partial pressure is sufficiently elevated, oxygen will pass into the blood stream even in the presence of reduced membrane permeability. This has been demonstrated in diffuse disease involving a major portion of both lungs.[44, 45] The same should be true if portions of a single lung are involved.

The oxygen tension of the pulmonary arterial blood is the same for both lungs. Its elevation due to bilateral oxygen breathing should not affect one lung more than the other as long as the pressure gradient between the alveolar oxygen tension and the pulmonary arterial oxygen tension remains the same on both sides of the membrane.

The fraction of the right ventricular output delivered to each lung is fixed within certain limits. The pressure in the two branches of the pulmonary artery is the same and the resistance to flow is greater in the diseased lungs under any circumstances. This increased resistance is, in great part, due to unalterable mechanical reduction of the vascular bed by thrombosis, fibrosis, and collapse. In the remaining arterioles and capillaries the resistance to flow may correspond to that of an area of similar magnitude in the well lung. As the output from the

right heart increases during exercise, the blood flow through the two lungs increases in proportion to the resistance to flow offered by the two lungs, that is, in the same proportion as the flow at rest.[37]

One may speculate that certain, as yet unexplained factors may be responsible for an increased blood flow through the diseased lung during oxygen breathing. Euler and Liljestrand[46] have measured pulmonary arterial pressure directly in anesthetized cats, and have noted an increased pressure following inhalation of gases with low oxygen tension, and a decreased pressure after pure oxygen breathing. The pulmonary arterial pressure effect of gases containing less oxygen than room air was confirmed in man by Motley and associates[47] and in isolated perfused lungs by Nisell.[48] If the pulmonary blood flow varies inversely to the pulmonary arterial pressure, then these observations may explain the mechanism whereby perfusion is adapted to alveolar ventilation in different parts of the lung. Indeed, this was demonstrated in rabbits by a bronchospirometric technique by Dirken and Heemstra.[49, 50] Unlike the results of the previous two investigations, peripheral arterial hypoxia was avoided and changes in differential pulmonary arterial flow could be ascribed solely to changes in composition of the inspired gas. Differential pulmonary blood flow was calculated from arterial blood analysis[49] or from relative oxygen uptake during brief intervals when both lungs were permitted to breathe the same gas.[50] Lowering of the oxygen content of the gas to one lung often reduced local circulation to less than half of the original value with a corresponding increase of blood flow through the other lung. A decrease of the alveolar oxygen tension of only 2.5 per cent reduced ipsilateral flow by 15 per cent. However, the adjustments required several hours. Rahn and Bahnson performed bronchospirometry in dogs and were able to obtain the gas content of the arterial blood, the respiratory quotient and the metabolic exchange rate for each lung.[51] From the data they calculated blood flow through each lung by the Fick principle. Nitrogen breathing of the left lung reduced its blood flow from approximately 45 to 25 per cent of the total cardiac output within ten minutes. The greatest shift of blood flow occurred within one to three minutes after initiation of unilateral nitrogen breathing. The rapid changes corresponded closely to results of anoxia experiments by Euler and Liljestrand, and Motley and associates.

These experiments suggest one of the mechanisms whereby the relative oxygen uptake of the diseased lung may be increased by an oxygen-enriched atmosphere. During ambient air breathing many areas of the diseased lung are underventilated with resulting low alveolar oxygen tension. According to the evidence presented, the resistance to blood flow in the diseased lung may be augmented by many areas of intense vasoconstriction. With a rise of alveolar oxygen tension due to oxygen breathing this resistance should decrease to a greater extent than the resistance of the normal lung whose vascular tone was not elevated during room air breathing. This unequal decrease of resistance may cause a relatively larger share of the pulmonary arterial blood to flow through the affected lung.

More experimental evidence will be required before this mechanism of local adaptation of blood flow to ventilation can be accepted. Lewis, Gorlin, and

Haynes[52] have recently subjected intact animals to breathing of oxygen-poor mixtures. They concluded that severe hypoxia does not alter pulmonary vascular resistance, on the contrary, it decreases this resistance and increases cardiac output. On the other hand, if the peripheral arterial oxygen saturation was maintained above 55 per cent, hypoxia resulted in decreased blood flow and increased pulmonary resistance. Motley and associates[47] made observations in normal man made anoxic. Dexter and associates[53] performed the corollary experiment. Pulmonary arterial pressure and cardiac output were measured in patients with pulmonary hypertension and chronic hypoxia due to emphysema and fibrosis. Breathing of oxygen-rich mixtures produced the expected reduction of pulmonary hypertension only in those patients who had an initially low cardiac output. Ferrer and associates[54] demonstrated similar changes when hypoxia was eliminated by treatment of the underlying pulmonary disease.

The relative increase of oxygen uptake by the diseased lung on breathing oxygen must be primarily due to alterations in alveolar gas composition resulting in a more favorable gradient between the alveolar and pulmonary arterial oxygen tensions. An increased blood flow through the diseased lung may occur although further evidence is needed before this mechanism can be removed from the realm of speculation.

SUMMARY

Previous work on ambient air spirometry was reviewed, and its application to bronchospirometry was discussed.

Three systems for ambient air breathing and continuous recording of respiratory excursions were described. The first permits constant room air breathing and recording of respiratory excursions, the second makes possible instantaneous switching to conventional oxygen rebreathing spirometry without interruption of recording, while the third allows for analysis of expired air after spirometry.

A bronchospirometer was described which contains twin drum-balloon systems for room air and conventional oxygen recording spirometry, as well as visible, low-resistance, respiratory valves and ventilographs for integration of differential ventilation.

Three normal volunteers were examined by ambient air bronchospirometry followed by conventional oxygen breathing. The relative oxygen uptake for the two lungs remained unchanged.

Twelve patients with moderate unilateral pulmonary functional impairment due to bronchogenic carcinoma, hemothorax, tuberculosis, and surgical collapse or resection were studied. There was no difference of relative oxygen uptake of the involved lung on changing from room air to oxygen breathing.

A third group of seven patients had severe unilateral pulmonary insufficiency due to far-advanced tuberculosis, hemofibrothorax, empyema, bronchogenic carcinoma with distal atelectasis, or congenital absence of one branch of the pulmonary artery. The mean oxygen uptake of the affected side was 3 per cent of the total on room air breathing and 12 per cent of the total on oxygen breathing.

It was concluded that conventional oxygen bronchospirometry gives an incorrect impression of the functional participation of greatly damaged lungs but does not significantly alter results of bronchospirometry in normal volunteers and patients with but slight unilateral pulmonary involvement.

The greater participation in oxygen uptake by the diseased lung on breathing an oxygen-enriched atmosphere was ascribed to an elevation of the oxygen tension in poorly ventilated alveoli sufficient to overcome the barrier of diseased alveolar membranes, and, in addition, pulmonary vasodilatation permitting a larger blood flow.

REFERENCES

1. Gaensler, E. A.: Bronchospirometry. I. Review of the Literature, J. Lab. & Clin. Med. **39**: 917, 1952.
2. Gaensler, E. A., Maloney, J. V., Jr., and Björk, V. O.: Bronchospirometry. II. Experimental Observations and Theoretical Considerations of Resistance Breathing, J. Lab. & Clin. Med. **39**: 935, 1952.
3. Gaensler, E. A., and Watson, T. R., Jr.: Bronchospirometry. III. Complications, Contraindication, Technique and Interpretation, J. Lab. & Clin. Med. **40**: 223, 1952.
4. Björkman, S.: Bronchospirometrie, eine klinische Methode die Funktion der menschlichen Lungen getrennt und gleichzeitig zu untersuchen, Acta Med. Scandinav. Supp. 56, 1934.
5. Gebauer, P. W.: A Twin Spirometer for Bronchospirometry, Dis. Chest **6**: 377, 1940.
6. Gaensler, E. A., and Watson, T. R., Jr.: Bronchospirometry. VI. Analysis of Results of 1,000 Examinations, In preparation.
7. D'Silva, J. L., and Mendel, D.: The Maximum Breathing Capacity Test, Thorax **5**: 325, 1950.
8. Whitehead, W. K., Whitman, N., and Shacter, B.: The Functional Capacity of the Contralateral Lung, Am. Rev. Tuberc. **47**: 173, 1943.
9. Wright, G. W., and Woodruff, W.: Bronchospirography. Ventilation and Oxygen Absorption of Normal and Diseased Lungs During Nitrogen Respiration in the Opposite Lung, J. Thoracic Surg. **11**: 278, 1941.
10. Uhlenbruck, P.: Beobachtungen zur rechtsventrikulären Herzinsuffizienz, Deutsch. Arch. f. klin. Med. **163**: 220, 1929.
11. Maloney, J. V., Jr.: Personal communications.
12. Gaensler, E. A., and Strieder, J. W.: Progressive Changes in Pulmonary Function after Pneumonectomy. The Influence of Thoracoplasty, Pneumothorax, Oleothorax and Plastic Sponge Plombage on the Pneumonectomy Side, J. Thoracic Surg. **22**: 1, 1951.
13. Gaensler, E. A., Frank, N. R., Patton, W. E., Smith, S. S., and Devney, R. E.: Modifications of the Open-Circuit Technique for Determination of Total Lung Capacity. Results of Duplicate and Multiple Studies. In preparation.
14. Gaensler, E. A.: Analysis and Critique of Pulmonary Function Studies, Bull. New England Med. Center **13**: 49, 1951.
15. Roth, H. P.: Apparatus for Determining Metabolism or Type of Respiration (Spirogram) with Facilities for Breathing of a Gas of Constant Composition, Boston, Thesis, 1939.
16. Sonne, C.: On the Movements in the Lungs During the Respiration. Survey of Problems and Description of Apparatus, Acta Med. Scandinav. **105**: 314, 1940.
17. Sonne, L. M., and Georg, J.: Changes in Resting Capacity (Functional Residual Air) of Lungs, Nord. med. (Hospitalstid.) **27**: 1395, 1945.
18. Sonne, L. M., and Georg, J.: Measurements of Respiratory Minute Volume, Nord. med. **42**: 1636, 1949.
19. Donald, K. W., and Christie, R. V.: A New Method of Clinical Spirometry, Clin. Science **8**: 21, 1949.
20. Georg, J.: Apparatus and Methods for the Estimation of Pulmonary Function, Scandinav. J. Clin. & Lab. Invest. **1**: 239, 1949.
21. Jansen, K., Knipping, H. W., and Stromberger, K.: Klinische Untersuchungen über Atmung und Blutgase, Beitr. z. Klin. Tuberk. **80**: 304, 1932.
22. Zaeper, G.: Zur Methodik der funktionellen Analyse von Atmung und Kreislauf, Beitr. z. Klin. Tuberk. **90**: 115, 1937.
23. Arnaud, J., Tulou, P., and Merigot, R.: L'Exploration de la Fonction Respiratoire, Paris: Masson et Cie., 1947, pp. 106-108.
24. Cournand, A., Richards, D. W., Jr., and Darling, R. C.: Spirography in Pulmonary Disease, Am. Rev. Tuberc. **40**: 487, 1939.
25. Nager, G.: Über das sogenannte Sauerstoffdefizit nach Uhlenbruck-Knipping, Schweiz. Ztschr. Tuberk. **4**, Supp. 1, 1947.

26. Anthony, A. J.: Funktionsprüfung der Atmung, Leipzig: Barth Verlag, 1937, p. 154.
27. Goralewski, G.: Zentralnervensystem und Anoxamie, Arbeitsphysiol., **9**: 94, 1935.
28. Benedict, F. G., Lee, R. C., and Strieck, F.: The Influence of Breathing Oxygen-Rich Atmospheres on Human Respiratory Exchange During Severe Muscular Work and Recovery from Work, Arbeitsphysiol. **8**: 266, 1934.
29. Herrald, F. J. C., and McMichael, J.: Determination of Lung Volume: Simple Constant Volume Modification of Christie's Method, Proc. Roy. Soc., London s.B. **126**: 491, 1939.
30. Bjerknes, W., and Scholander, P. F.: Method for Continual Air-breathing in Closed-Circuit Apparatus, Scandinav. Arch. f. Physiol. **79**: 164, 1938.
31. Eckman, M., and Barach, A. L.: Horizontal Respiratory Graphs by Automatic Feed of Oxygen into a Basal Metabolism Apparatus, Proc. Soc. Exper. Biol. & Med. **36**: 138, 1937.
32. Anthony, A. J., and Rohland, R.: Ein Spirograph mit automatischer Regelung des Sauerstoffgehaltes, Ztschr. f. d. ges. exper. Med. **106**: 555, 1939.
33. Rossier, P. H., and Wiesinger, K.: Stabilizator für die Sauerstoffspannung im geschlossenen Spirometriesystem, Schweiz. Ztschr. Tuberk. **6**: 17, 1949.
34. Whitfield, A. G. W., Waterhouse, J. A. H., and Arnott, W. M.: The Total Lung Volume and its Subdivisions. A Study in Physiological Norms. I. Basic Data, Brit. J. Soc. Med. **4**: 1, 1950.
35. Willard, H. N., and Wolf, G. A., Jr.: A Source of Error in the Determination of Basal Metabolic Rates by the Closed-Circuit Technic, Ann. Int. Med. **34**: 148, 1951.
36. Tulou, P.: La broncho-spirometrie avec sonde souple. Méthode d'éxploration fonctionelle des poumons séparés, Rev. de la Tuberc. **11**: 543, 1947.
37. Björkman, S., and Carlens, E.: The Lung Function During Rest and Exercise in Lung Disease. A Bronchospirometric Study, Acta Med. Scandinav. Supp. 259, 1951, pp. 63-68.
38. Reichert, P.: Oxygen Utilization as an Index of Respiratory Efficiency. A Clinical Device for its Determination, J. Aviation Med. **7**: 63, 1936.
39. Patton, W. E., Watson, T. R., Jr., and Gaensler, E. A.: Pulmonary Function Before and at Intervals After Surgical Decortication of the Lung, Surg., Gynec. & Obst. 1952.
40. Madoff, I. M., Gaensler, E. A., and Strieder, J. W.: Congenital Absence of the Right Pulmonary Artery. Diagnosis by Angiocardiography and with Cardio-Respiratory Studies, New England J. Med. 1952.
41. Fowler, W. S., and Comroe, J. H., Jr.: Lung Function Studies. I. The Rate of Increase of Arterial Oxygen Saturation During the Inhalation of 100 Per Cent Oxygen, J. Clin. Investigation **27**: 327, 1948.
42. Comroe, J. H., Jr., and Dripps, R. D.: The Oxygen Tension of Arterial Blood and Alveolar Air in Normal Human Subjects, Am. J. Physiol. **142**: 700, 1944.
43. Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E.: An Experimental Analysis in Man of the Oxygen Pressure Gradient from Alveolar Air to Arterial Blood During Rest and Exercise at Sea Level and at Altitude, Am. J. Physiol. **147**: 199, 1946.
44. Wright, G. W., and Filley, G. F.: Pulmonary Fibrosis and Respiratory Function, Am. J. Med. **10**: 642, 1951.
45. Motley, H. L., and Tomashefski, J. F.: Effect of High and Low Oxygen Levels and Intermittent Positive Pressure Breathing on Oxygen Transport in the Lungs in Pulmonary Fibrosis and Emphysema, J. Applied Physiol. **3**: 189, 1950.
46. Euler, v. U. S., and Liljestrand, G.: Observation on the Pulmonary Arterial Blood Pressure in the Cat, Acta Physiol. Scandinav. **12**: 301, 1946.
47. Motley, H. L., Cournand, A., Werko, L., Himmelstein, A., and Dresdale, D.: The Influence of Short Periods of Induced Acute Anoxia Upon Pulmonary Artery Pressure in Man, Am. J. Physiol. **150**: 315, 1947.
48. Nisell, O. I.: The Action of Oxygen and Carbon Dioxide on the Bronchioles and Vessels of the Isolated Perfused Lungs, Acta Physiol. Scandinav. **21**, Supp. 73, 1950.
49. Dirken, M. N. J., and Heemstra, H.: Alveolar Oxygen Tension and Lung Circulation, Quart. J. Exper. Physiol. **34**: 193, 1947/8.
50. Dirken, M. N. J., and Heemstra, H.: The Adaptation of the Lung Circulation to the Ventilation, Quart. J. Exper. Physiol. **34**: 213, 1947/8.
51. Rahn, H., and Bahnson, H. T.: Simultaneous Determination of Blood Flow Through Each Lung, Fed. Proc. **9**: 102, 1950.
52. Lewis, B. M., Gorlin, R., and Haynes, F. W.: Am. J. Physiol., to be published.
53. Dexter, L., Whittenberger, J. L., Gorlin, R., Lewis, B. M., Haynes, F. W., and Spiegl, R. J.: The Effect of Chronic Pulmonary Disease (Cor Pulmonale and Hypoxia) on the Dynamics of the Circulation in Man, Tr. A. Am. Physicians **64**: 226, 1951.
54. Ferrer, M. I., Harvey, R. M., Cathcart, R. T., Webster, C. A., Richards, D. W. Jr., and Cournand, A.: Some Effects of Digoxin Upon the Heart and Circulation in Man, Circulation **1**: 161, 1950.

SPECTROPHOTOMETRIC DETERMINATION OF OXYHEMOGLOBIN SATURATION AND OXYGEN CONTENT OF BLOOD

ALBERT ROOS, M.D., AND J. A. RICH, B.S.
ST. LOUIS, MO.

SEVERAL methods have recently been published for the determination of oxygen in blood by means of spectrophotometry. Thus, Nahas[1] described a technique using a specially designed cell of 0.1 mm. optical path. Hickam and Frayzer[2] used 5 mm. cuvettes; their method was primarily designed for the measurement of differences in oxygen content between two blood samples of the same total hemoglobin content. Other recent and older publications on the same subject have been reviewed before.[2, 3]

The present method foregoes the need for a special cuvette, and an unlimited number of samples can be examined using the standard equipment of the monochromater.

METHODS

Principle.—The blood is hemolyzed and diluted in a closed system with a deaerated saponin solution of such high alkalinity that the oxyhemoglobin dissociation curve is maximally shifted to the left. Any tendency for the saturation to diminish, due to the dilution and its resultant fall in oxygen tension, will thus be opposed. In fact, there may even be a tendency for the saturation to rise, though a significant increase is prevented because of lack of oxygen in the system.*

The optical density of the blood solution is then read at two wave lengths, and from these two values and the extinction coefficients of oxyhemoglobin and reduced hemoglobin at the same wave lengths the oxyhemoglobin saturation can be calculated. Neither the dilution nor the optical path need be exactly known. The purpose of the dilution is to allow enough light to be transmitted at the selected wave lengths and optical path. Thus the need for excessively thin cuvettes[1] is circumvented. To find the oxygen content of the blood sample a separate measurement is performed for the determination of total hemoglobin.

Methods.—(a) *Blood collection:* 1 to 5 ml. of blood are anaerobically collected in a lightly greased syringe, the dead space of which has been filled with heparin solution. Immediately after the blood a small amount of mercury is drawn up, the needle removed, and the syringe closed with a two-way B.D. stopcock. Gentle tilting will allow intimate mixing of heparin with the blood.

(b) *Preparation of alkaline saponin solution:* 21.12 Gm. of boric acid and 13.65 Gm. of NaOH are dissolved in 1 liter of distilled water (0.342M borate buffer). At 0.1M and 30° C., this buffer has a pH of 10.82.[4] It can be kept for several weeks, preferably in the refrigerator. On the day of the analysis a 5 per cent saponin (Eastman Kodak Company) solution in this buffer is made up and deaerated in the Van Slyke-Neill manometric apparatus in the conventional way. One drop of caprylic alcohol is added to prevent foaming. The solution is taken up in a syringe with an adapter. The latter consists of a three-way B.D. stopcock attached through a short length of thick-walled rubber tubing to 3 to 4 inches of

From the Laboratory of Thoracic Physiology, Department of Surgery, and the Department of Physiology, Washington University School of Medicine.

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*This principle was used also by K. Kramer and J. N. Nilson (1940, unpublished data).

glass tubing with tapered end fitted with a rubber tip (we used the end of a discarded pipette). The dead space of the syringe is filled with mercury. The tip is pressed firmly into the cup through some mercury. The first few milliliters of the solution in the Van Slyke machine are discarded through the side opening of the B.D. cock. A fresh solution has to be made up every day.

(c) *Dilution of the blood, transfer to cuvette, and reading:* The saponin and blood syringes are attached to each other by means of their stopcocks, both syringes lying on the table. After the dead space of the cocks is filled with solution and blood, the plunger of the blood syringe is slowly withdrawn and a volume of saponin solution one to two times that of the blood is transferred. Both syringes are closed, the blood syringe with its stopcock detached, and its contents shaken for a minute after which a 3-inch size 22 spinal needle bent at a 30 degree angle is attached. One to two milliliters of the blood solution are discarded through the needle and its tip lowered and kept at the very bottom of the cuvette which is slowly filled. No oil is necessary as the light passes through the lower part of the cuvette which is separated from the air by an appreciable column of fluid. Readings should be made within two to three minutes at 600 and 505 mμ. The latter wave length is an isobestic point for oxy- and reduced hemoglobin. As blank a similar cuvette is filled with an identical solution, replacing blood by water.

(d) *Spectrophotometer and cuvette:* Our studies were carried out on a Model B Beckman spectrophotometer using a slit width of 0.1 to 0.2 mm. and the blue sensitive phototube. Under these conditions the effective band width is 2 to 3 mμ. Any monochromater with adequate resolving power should be suitable. The optical path of the cuvette is about 1 mm., obtained by placing a 9 mm. spacer in a 10 mm. cuvette. As knowledge of the exact length of the optical path is not required (see calculations), plastic cuvettes with homemade plastic spacers are quite satisfactory.

(e) *Determination of extinction coefficients:* Fully saturated blood (equilibrated in a Barcroft flask) is divided into two parts. On one part a gasometric oxygen analysis is performed after which the appropriate deduction is made for dissolved oxygen; the second part is diluted with exactly equal volume of the saponin solution (not necessarily evacuated) after which its optical density at the two wave lengths is determined in a cuvette of exactly known optical path (about 1 mm.). To the remainder of the second part powdered $Na_2S_2O_4$, 0.1 mg. per milliliter, is added, after which the optical densities of this reduced hemoglobin solution are determined. The average of a series of such determinations produces the average value for the extinction coefficients; that is, the optical density per mM/L. per 10 mm. optical path. The values were found to be the same whether the blood was diluted one-half or one-third with saponin solution.

(f) *Determination of total hemoglobin content:* This is required if the oxygen content, rather than oxyhemoglobin saturation of the sample, is desired. We used the conventional technique, diluting (in duplicate) 20 cu. mm. of blood in 10 ml. of distilled water, to which was added 1 drop of concentrated ammonia and measuring the optical density at 540 mμ in a calibrated 10 mm. cuvette. A previously obtained calibration curve (against gasometric analysis) produced a straight line through the origin.

Calculations: According to Beer's Law:

(1) $O.D._{505} = \epsilon_{505} d(c_{HbO_2} + c_{Hb})$ and
(2) $O.D._{600} = \epsilon_{600HbO_2} dc_{HbO_2} + \epsilon_{600Hb} dc_{Hb}$, where
O.D. = optical density of the unknown sample
ϵ = extinction coefficient (optical density per mM/L. per 1 cm. optical path)
c = concentration (mM/L.)
d = optical path (cm.)

Also

$$\text{Per cent oxyhemoglobin saturation} = \frac{c_{HbO_2}}{c_{HbO_2} + c_{Hb}} \times 100$$

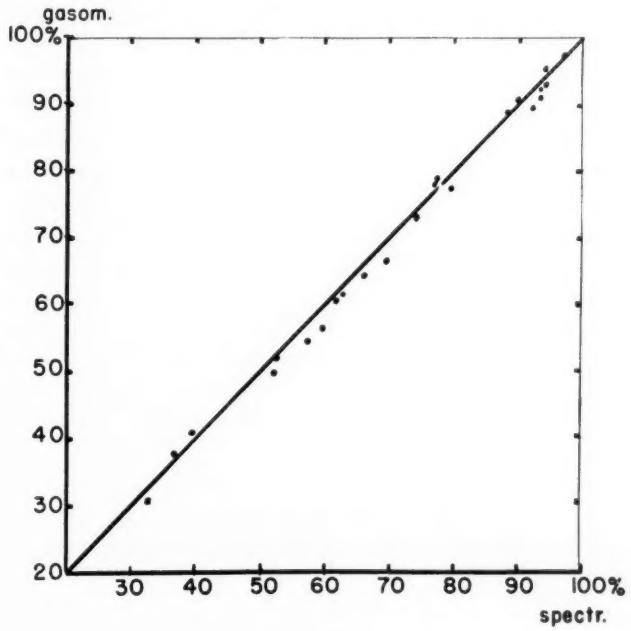
With the use of (1) and (2) this becomes

$$(3)\ \text{Per cent } HbO_2 \text{ saturation} = \left[\frac{\epsilon_{600Hb}}{\epsilon_{600Hb} - \epsilon_{600HbO_2}} - \left(\frac{O.D._{600}}{O.D._{505}} \times \frac{\epsilon_{505}}{\epsilon_{600Hb} - \epsilon_{600HbO_2}}\right)\right] \times 100$$

Once the extinction coefficients have been determined, equation (3) can be reduced to

$$\text{Per cent } HbO_2 \text{ saturation} = \left(K_1 - \frac{O.D._{600}}{O.D._{505}} \times K_2\right) \times 100$$

where K_1 and K_2 are optical constants. As only the ratio of the two optical densities of the unknown sample appears in the equation, neither knowledge of the exact amount of dilution nor that of the exact length of the optical path is required.



RESULTS

The average values for the extinction coefficients of human blood using the apparatus and technique described were as follows:

$$\epsilon_{505} = 5.60, \quad \epsilon_{600Hb} = 3.24, \quad \epsilon_{600HbO_2} = 0.864$$

Equation (3) became therefore:

$$\text{Per cent } HbO_2 \text{ saturation} = \left(1.365 - \frac{O.D._{600}}{O.D._{505}} \times 2.36\right) \times 100$$

Twenty-four blood samples with saturations ranging from 30 to 100 per cent were analyzed spectrophotometrically and by means of the conventional manometric technique. Duplicate gasometric content determinations checked to 0.05 vol. per cent. The results are plotted in Fig. 1. The average of the saturations obtained by optical means was 1 per cent higher; the standard deviation of the differences was 1.5 per cent. The mean difference between

optical and gasometric oxygen capacity determinations was 0.06 vol. per cent. Both of the latter analyses were done on blood equilibrated with room air in a flask for about twenty minutes.

DISCUSSION

When one or two parts of the saponin buffer are mixed with one part of blood, the pH of the resulting blood solution becomes about 9.70. At pH 9.0 the maximal effect of alkalinity upon the position of the dissociation curve is reached.[5] Roughton and Root[6] studied the shape of the oxygen dissociation curve of human 1:5 diluted blood solution at 25° C. and at pH of about 8.6. The oxygen pressure for 30 per cent saturation was about 2, for 50 per cent about 3, for 90 per cent about 6 mm. Hg.[7] Native human blood at 30 per cent saturation and pH 7.42 will have its original pO_2 of 16 reduced to 8 or 5 mm. Hg by respectively 1:1 or 1:2 dilution with oxygen-free saponin solution. These pressures are still in excess of the pO_2 at pH 8.6 at the same saturation, so that a trace of oxygen will be taken up by the hemoglobin. At average normal capacity the saturation would increase by at the most 0.2 per cent, at higher capacities by less than that. Similarly, at 90 per cent saturation the pO_2 will be reduced from 57 to 28 or 19 mm. Hg, resulting in a rise of at the most 0.6 per cent saturation. Thus only a part of the difference between optical and gasometric measurements can be explained on this basis. An additional fact leading to a discrepancy in the same direction is the increase of 1 or 2 per cent in oxygen capacity during equilibration, leading to erroneously low saturation values by the gasometric method.[8] These two factors may explain the slight difference between the means of the two groups of data.

It finally should be pointed out that the method described is based on the assumption of a two component system of hemoglobin and oxyhemoglobin. It neglects the small amounts of carboxyhemoglobin and of "inactive hemoglobin" that may be present.[8, 9] This is a feature common also to previous spectrophotometric methods, including that of Drabkin.[10]

SUMMARY

A spectrophotometric technique is described for the measurement of oxyhemoglobin saturation and oxygen content of blood. In a series of twenty-four determinations the mean difference between parallel gasometric saturation studies was +1 per cent, with a standard deviation of the differences of 1.5 per cent.

REFERENCES

1. Nahas, G. G.: Spectrophotometric Determination of Hemoglobin and Oxyhemoglobin in Whole Hemolyzed Blood, Science **113**: 723, 1951.
2. Hickman, J. B., and Frayzer, R.: Spectrophotometric Determination of Blood Oxygen, J. Biol. Chem. **180**: 457, 1949.
3. Wood, E. H.: Blood Gas Transport, Ann. Rev. Physiol. **14**: 235, 1952.
4. Clark, W. M.: Determination of Hydrogen Ions, ed. 3, Baltimore, 1928, Williams and Wilkins Company.
5. Hastings, A. B., Van Slyke, D. D., Neill, J. M., Heidelberger, M., and Harington, C. R.: The Acid Properties of Reduced and Oxygenated Hemoglobin, J. Biol. Chem. **60**: 89, 1924.

6. Roughton, F. J. W., and Root, W. S.: The Estimation of Small Amounts of Carbon Monoxide in Air, J. Biol. Chem. **160**: 135, 1945.
7. Forbes, W. H., and Roughton, F. J. W.: The Equilibrium Between Oxygen and Haemoglobin, J. Physiol. **71**: 229, 1931.
8. Roughton, F. J. W., Darling, R. C., and Root, W. S.: Factors Affecting the Determination of Oxygen Capacity, Content and Pressure in Human Arterial Blood, Am. J. Physiol. **142**: 708, 1944.
9. Van Slyke, D. D., Hiller, A., Weisiger, J. R., and Cruz, W. O.: Determination of Carbon Monoxide in Blood and of Total and Active Hemoglobin by Carbon Monoxide Capacity. Inactive Hemoglobin and Methemoglobin Contents of Normal Human Blood, J. Biol. Chem. **166**: 121, 1946.
10. Drabkin, D. L., and Schmidt, C. F.: Observations of Circulating Blood in Vivo and the Direct Determination of the Saturation of Hemoglobin in Arterial Blood, J. Biol. Chem. **157**: 69, 1945.

USE OF AN ARTIFICIAL KIDNEY

III. Current Procedures in Clinical Hemodialysis*

William P. Murphy, Jr., M.D., Roy C. Swan, Jr., M.D.,**
Carl W. Walter, M.D., John M. Weller, M.D., and
John P. Merrill, M.D.,*** Boston, Mass.

INTRODUCTION

THE technique of circulating blood extracorporeally for the purpose of dialyzing out metabolites present in abnormally high concentrations in body fluids in acute and chronic renal failure is being applied clinically in several centers today. The procedure presents problems in clotting, hemolysis, bleeding, exsanguination, plethora, adequacy of blood flow through the apparatus, leakage, sepsis, air embolism, electrolyte and water balance, temperature control, and comfort of the patient. Experience in this laboratory with these problems has led to technical modifications which may be useful to those engaged in extracorporeal blood circulation in general and hemodialysis in particular.

Since earlier reports of technical and clinical experience with a modified Kolff artificial kidney,[1-3] hemodialysis has been applied 165 times in 110 patients, bringing to 225 the number of times the procedure has been used clinically in this hospital over a three and one-half year period. No death attributable to this procedure has occurred during this three and one-half year experience, although hemodialysis has been accomplished in three patients under 4 years of age, fourteen patients over 70 years of age, and in patients with such serious problems as extensive third-degree burns, peritonitis, shock, coma, congestive heart failure, profound anemia, and recent myocardial infarction. This report deals with technical changes designed to make the procedure of clinical hemodialysis with the modified Kolff hemodialyzer safer, simpler, more efficient, and less costly in time and material.

MATERIALS AND METHODS

Technical Modification for Clinical Hemodialysis.—The modified Kolff hemodialyzer (Fig. 1) receives blood from the radial artery through a rotating coupling to a dialyzing membrane consisting of a long cellophane tube wound spirally on a drum rotating in a bath. From the cellophane tubing blood passes through a distal rotating coupling and return pump to a reservoir, clot-catcher, and bubble trap from which the blood returns to a forearm vein. It is obvious that the dialyzing area should be large and the volume of blood in the apparatus small in order to increase dialyzing efficiency and minimize the volume of blood required to

From the Departments of Medicine and Surgery, Peter Bent Brigham Hospital and Harvard Medical School.

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fill the apparatus prior to dialysis. Blood should contact hemorepellent surfaces only. For ease and speed of assembly and to reduce opportunity for contamination, the number of parts and connections in the circuit should be minimal. These parts should withstand autoclaving after assembly without distortion of finely machined surfaces. With these aims in mind, the following changes have been made in the apparatus previously described.[1]

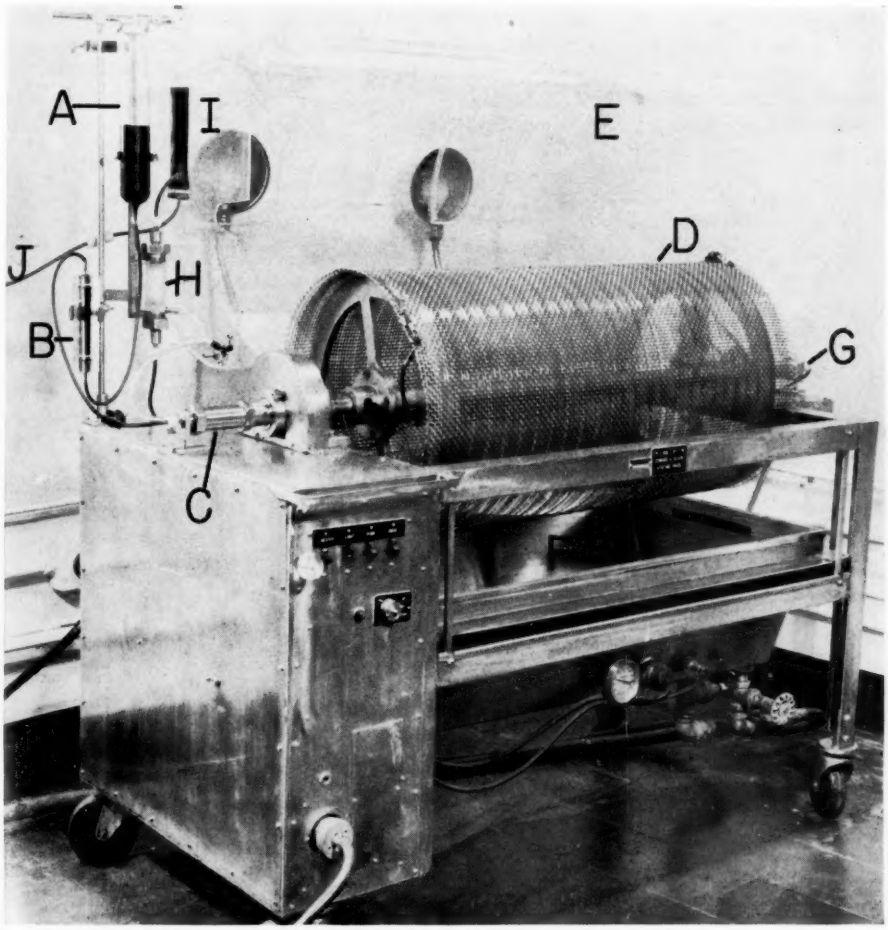

Fig. 1.—Modified Kolff hemodialyzer. *A*, Filling burette (replaced by arterial lead when dialysis begins); *B*, flow meter; *C*, proximal rotating coupling; *D*, Cellophane on rotating drum; *E*, lucite hood; *F*, bath in lowered position; *G*, distal rotating coupling; *H*, return pump; *I*, reservoir, clot and bubble trap; *J*, lead to vein.

Tubing and Cannulae.—The nonautoclavable polyethylene cannulae, rubber connectors, and large bore plastic tubing have been replaced by smaller (3/16 inch internal diameter, the diameter of all other orifices in the apparatus) polyvinyl chloride tubing. The arterial and venous leads are prepared in advance by drawing out the ends into appropriately sized cannulae after softening and deplasticizing in mineral oil at 175° C. for a few minutes. The cannulae are deplasticized to a hardness sufficient to prevent crushing by ligatures. A gum rubber sleeve (1 inch long, ¼ inch internal diameter 1/16 inch wall) is slipped over the end of each cannula just before use to provide a self-sealing area for blood sampling and intravenous medication. Cannulae are made sufficiently long to allow the surgeon to trim them

to the desired length and cut a suitable bevel. Excessively long cannulae will introduce excessive pressure drop and decrease blood flow. Cannulae with sharp or ragged edges may strip the intima and become blocked.

Couplings.—The rotating couplings (Fig. 2) are lined with Kel-F,* an extremely stable plastic resisting distortion during autoclaving and wear during the operation. The plastic parts are encased in a stainless steel shell revolving in a bronze bushing. The width of the rotating plastic face has been considerably reduced (9/32 inch to 1/32 inch) to increase surface loading. Blood leakage at the coupling has been completely eliminated. In order to stabilize the rotating member of the coupling, it has been lengthened, its bearing surfaces separated to approximately three and one-half times its diameter, and the loading spring replaced by a beryllium copper spring washer.

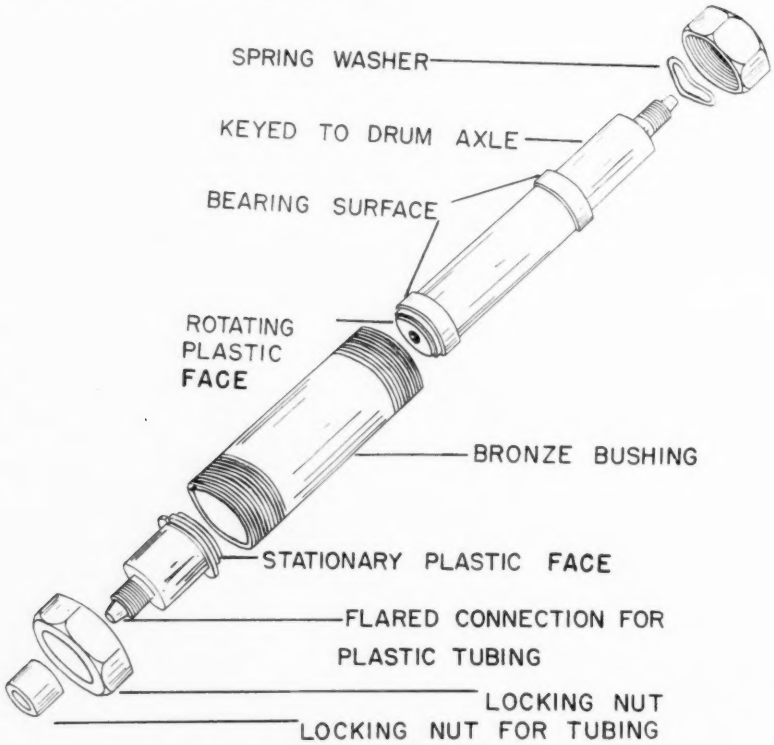

Fig. 2.—Exploded view of rotating coupling.

Connections.—3/16 inch interval diameter polyvinyl tubing runs through the hollow axle of the drum and connects the coupling with the cellophane. A ½ inch length of ¼ inch I.D. polyvinyl tubing slipped over one end and cemented with cyclohexanone gives a firm autoclavable anchor for tying the cellophane tubing over the polyvinyl tubing with two ¼ inch cotton tape ties. The polyvinyl tubing is attached to all parts with flare-type connections (Fig. 4, *D*), the tubing being pressed over a slight flare and held in place with a locking nut. This connection gives a uniform lumen with minimal turbulence.

Cellophane.—138 feet of 23/32 inch diameter lay-flat cellophane tubing (22,000 sq. cm. of dialyzing area) is wound tightly about the drum. Any slack in winding or stretch during operation accumulates at the distal end of the drum where it is taken up by a spring-loaded support rotating about the main axle. The cellophane area above the surface of the bath is

*A tetrafluoroethylene plastic.

effective for dialysis because of the considerable amount of bath fluid which is carried up with the drum and drains back to the bath when the drum rotates at 26 r.p.m. Complete immersion of the drum, if technically possible, would destroy the pumping action of the helically wound cellophane loops. Considerably less than the usual extent of drum immersion still results in efficient dialysis, as is shown in Table I.

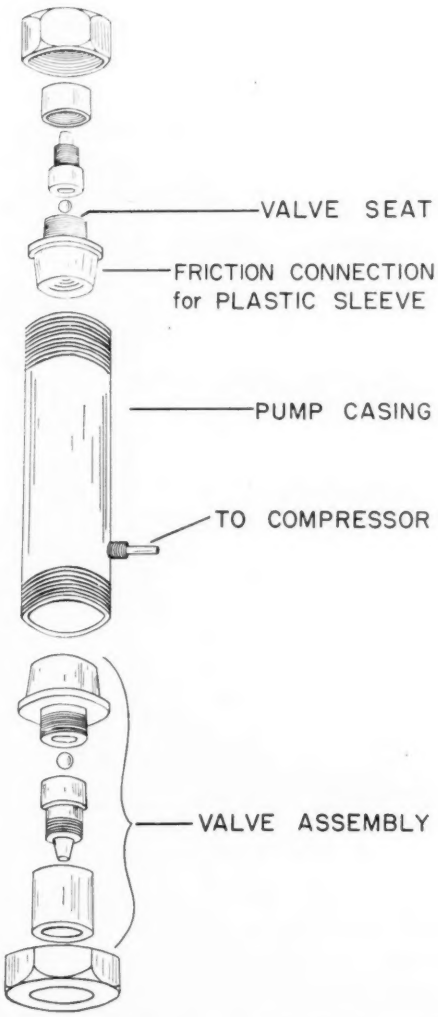

Fig. 3.—Exploded view of return pump. The flexible plastic tubing lying within the pump casing has been omitted from this drawing.

Return Pump.—Three forces propel blood through the apparatus. Arterial pressure serves only to propel blood through the arterial lead, flow meter, and proximal rotating coupling to the first cellophane loop. Thereafter, blood is propelled, independent of arterial pressure, by (a) the Archimedes screw principle of the helically wound cellophane tube, and (b) a pump (Fig. 3) returning blood from the terminal loop through a sealed clot and air bubble trap to a peripheral vein. The return pump is a flexible polyvinyl tube 1 inch in diameter surrounded by a rigid Kel-F casing with a check valve at each end. The tube

is alternately distended and collapsed by negative and positive pressure from a modified refrigerator-type compressor, stroke 1½ inch, bore 1½ inch, in phase with the rotating drum and begins suction when the distal end of the Cellophane tube is in its lowest position and thus full of blood. Any continuously acting pump, like the Beck pump, is unsuitable since, by creating suction when the terminal Cellophane loop is relatively empty of blood, it tends to draw air in through the interface of the distal rotating coupling. Such does not occur with a correctly synchronized, intermittently acting pump such as the one described. The pump parts are constructed of stainless steel, Kel-F, and polyvinyl chloride. Blood contacts hemorepellent surfaces only.

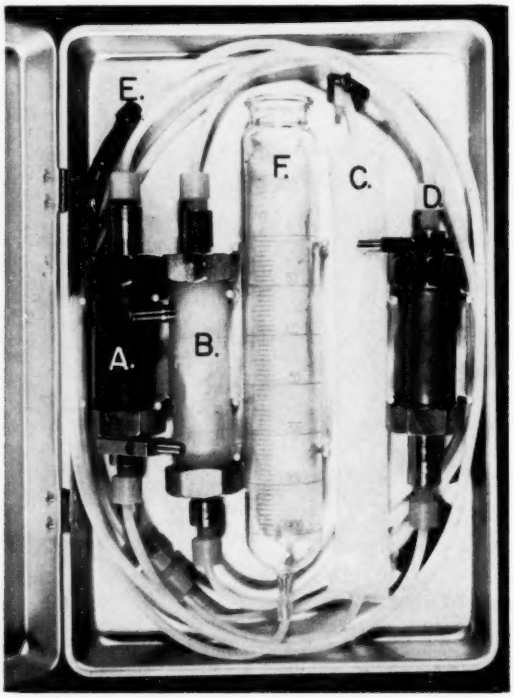

Fig. 4.—Autoclavable kit. *A*, rotating coupling; *B*, return pump; *C*, plastic reservoir, clot and bubble trap; *D*, Nylon tubing connector; *E*, wrapped end for Cellophane connection; F. filling and rinsing burette.

Clot and Bubble Trap.—Blood from the return pump enters a venous reservoir serving also as a clot and bubble trap. This is constructed of 1 inch diameter (0.032 inch wall) polyvinyl chloride tubing heat sealed at the ends. Glass beads act as clot catchers. The 3/16 inch tube at the top is clamped off when a satisfactory blood level and air cushion are established. Through this opening blood or air can be added or removed from the system. This arrangement affords a self-regulating positive pressure venous return since increased inflow from the pump tends to increase pressure within the reservoir, while reduced inflow results in progressively decreasing outflow as the pressure in the reservoir falls. The venous cannula is connected to the discharge side of the trap.

Autoclavable Kit.—All the above parts are autoclavable. The rigid plastic parts (connectors, couplings, pump valves, and casing) are made of Nylon or Kel-F and are reused. The polyvinyl parts are disposable. Except for the Cellophane and the flow meter, all the blood conducting parts, together with a glass burette for rinsing and filling the apparatus before dialysis, are assembled and autoclaved in an 11 inch by 16 inch aluminum container.

TABLE I. EXTRACTION RATIOS AT VARYING DEGREES OF DRUM IMMERSION

	% OF DRUM CIRCUMFERENCE IMMERSED	UREA EXTRACTION RATIO
A. Bath fully raised	40%	90%
B. Bath raised half-way	25%	88%
C. Bath touching	12%	85%

Osmolarity of urea solution and glucose bath solution = 33 mos. per liter

Urea solution = 89.5 mg. per cent urea nitrogen

Flow = 200 ml. per minute

Urea solution was dialyzed against an isosmotic glucose bath at three levels of drum immersion. Urea extraction ratio is the per cent urea removal in one passage through the apparatus and is an average of two ratios determined at each level of drum immersion: (a) 92 per cent, 88 per cent, (b) 87 per cent, 89 per cent, (c) 84 per cent, 86 per cent, Urea was determined in duplicate by urease aeration method of Van Slyke and Cullen.[4] Variation of averages of duplicates is 0.33 mg. per cent.

Flowmeter.—It is desirable to have a continuously indicating flow meter on the arterial side since the Cellophane tubing can be greatly distended if arterial flow exceeds venous flow for any length of time, and the patient made plethoric if arterial flow slows or stops. A flow meter on the venous side does not promptly reflect changes in arterial flow because blood requires about three minutes to pass through the apparatus. Of several types of flow meters considered, a simple rotameter seems currently most practical, giving an accuracy (within 15 per cent) sufficient for clinical purposes. We are using a lucite rotameter* sterilized by soaking eighteen hours in 1:1,000 aqueous Zephiran R and inserted between the arterial cannula and proximal rotating coupling. The addition of the rotameter to the apparatus has not resulted in clotting or hemolysis. A rough calibration chart has been prepared by a mass plot of two or more calibrations taken during each dialysis on several patients with widely varying hematocrits.

Bath.—The composition of the bath is indicated in Table II. Minor changes are made depending upon the clinical situation. Calcium and magnesium concentrations have been reduced to approximately match ionized calcium and magnesium concentrations in normal plasma. Except for calcium chloride, all chemicals are added in the solid form as the bath is being brought up to the 100 liter mark with tap water. The bath is then raised until the lower half of the drum is immersed, and a water seal is made between the bath and the lucite hood covering the drum.

Equilibration of the bath with 5 per cent carbon dioxide 95 per cent oxygen mixture theoretically reduces the pH from above 8.0 to 7.4. In practice, however, a greater concentration of carbon dioxide is required because the volume of air under the hood which must be displaced by the carbon dioxide mixture is over 100 liters, and because some leakage of gas occurs around the openings in the hood for the drum axle. Since the bath fluid is well agitated by the rotating drum and continuously being carried up over the drum as a thin film, admitting the carbon dioxideoxygen through a bubbler in the bath offers little advantage over a simple connection in the hood. Change in the bath pH during equilibration with 10 per cent carbon dioxide 90 per cent oxygen mixture is shown in Fig. 5. Calcium chloride, previously weighed out in individual paper containers and stored in a desiccant-containing canister, is dissolved in approximately 50 ml. of warm water and added through one of the apertures in the plastic hood after the bath has been equilibrated for fifteen minutes with carbon dioxide.

TABLE II. BATH COMPOSITION

	GMS/100 L	MOS/L	MEQ/L
NaCl	660	226	113
$NaHCO_3$	225	54	26.8
KCl	30	8.0	4.0
$MgCl_2 \cdot 6H_2O$	10	1.5	1.0
$CaCl_2 \cdot 2H_2O$	18.5	3.8	2.5
Glucose	200	10.0	$Na^+ = 140$
		303.3	$Cl^- = 120$

*Furnished by Mr. Harry Barker of the Westinghouse Electric Corporation.

In practice we no longer attempt to match bath osmolarity with plasma osmolarity in each patient by the addition of a calculated amount of glucose. The bath is made up to osmolarity equivalent to normal plasma. No large or consistent weight change results during dialysis.

Following dialysis the stainless steel bath tank and drum are washed with tap water. The use of detergents and antimicrobial agents has been eliminated.

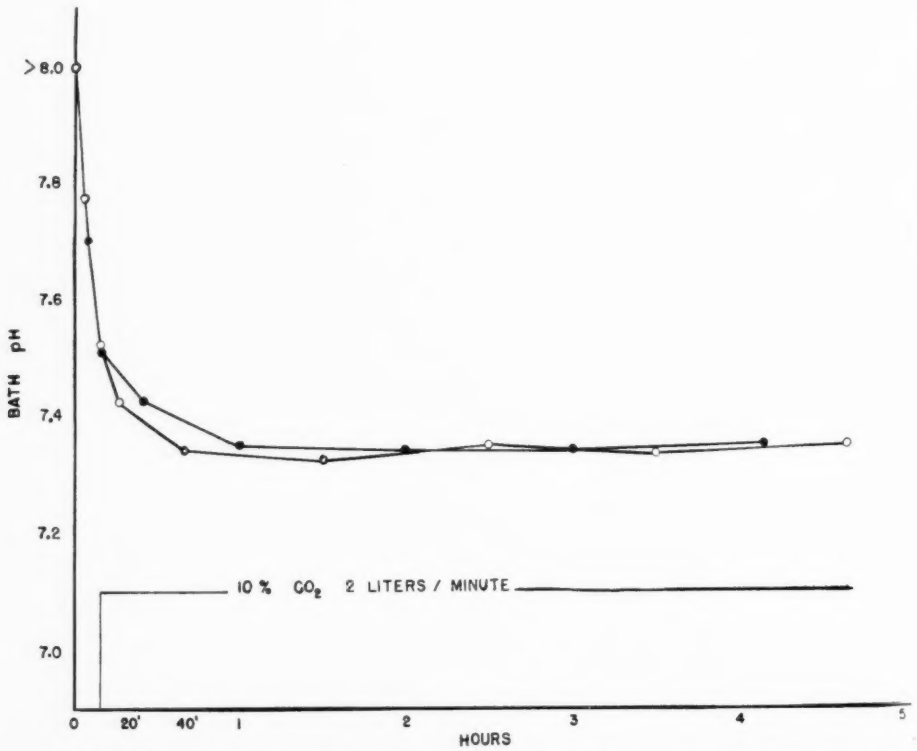

Fig. 5.—Change in bath pH during equilibration with 10 per cent carbon dioxide at 15 L per minute for ten minutes followed by 2 L per minute thereafter. The pH of bath was determined at 38.5° C. by the method of Hastings and Sendroy.[5]

Procedure.—Clotting time is determined, and 2 pints of blood are typed and cross-matched on the day before dialysis. On the morning of dialysis, the patient is sedated and weighed. Food is withheld. The autoclaved parts are attached to the machine. The Cellophane, which has been previously soaked overnight in water on a reel to remove the glycerine softening agent and boiled in two thirty-minute washes in an automatically timed bath, is wound on the drum. Fifty milliliters of bank blood diluted with saline are run in from the filling burette to test the system for leaks. The system is then flushed with 3 liters of saline and filled with 500 to 700 ml. of citrated bank blood. The bath is made up and kept at 39° C.

Meanwhile, the surgeon, having exposed the radial artery and a forearm vein, cannulates the vein, and through the cannula injects an appropriate amount of heparin, usually 100 mg. He then cannulates the artery and collects arterial samples for chemical analysis. The venous and arterial leads are allowed to fill with blood, are connected to the apparatus, and dialysis begins. Adjustable clamps on arterial and venous leads balance inflow and outflow. Distention of the terminal cellophane loop and change in blood level in the clot and air

bubble trap are early indications of imbalance between arterial and venous flow. Venous flow frequently lags behind arterial flow at the beginning of dialysis because cool blood in the return tubing may cause a transient venospasm. This can be relieved by the local application of heat. Thereafter venous flow improves and arterial flow is usually the limiting factor.*

Arterial flow depends in part upon the caliber, length, and position of the cannula, which in turn depend upon the nature of the vessel and the surgical skill employed. A 1½ to 2 cm. incision over artery and vein, the insertion of an arterial cannula with a 1 to 2 mm. inside diameter, and a venous cannula with a 2 to 3 mm. inside diameter will usually produce a 150 to 300 ml. per minute blood flow, a desirable range for the procedure. The apparatus is capable of handling 400 ml. per minute. At flows less than 100 ml. per minute dialysis is inefficient and clotting may occur. Hemodialysis is continued for six hours.

Clotting Hemolysis and Pyrogen Reactions.—Under the above circumstances there is no hemolysis as observed by hourly hematocrits. On a few occasions, using bank blood older than two weeks, we have observed very slight hemolysis in the first half-hour, the plasma pigment diminishing progressively thereafter. If the flow is greater than 100 ml. per minute, 100 mg. of heparin initially and 10 mg. hourly satisfactorily prevent clotting and does not result in seepage around cut-down sites. We have not found a close correlation between clotting time and tendency to clot in the apparatus, and in practice we no longer follow clotting time during dialysis. We have on occasion discontinued heparin administration after the second hour (120 mg.) and have not observed clotting during the subsequent four hours. It is our feeling, however, that less than 150 mg. of heparin for the six-hour period will not uniformly prevent clotting and will effect merely a slight saving of heparin at the possible expense of wasting much time and opportunity if clotting should stop the dialysis. Four mild pyrogen reactions have been observed in the last 165 runs.

Antibiotics.—We continue to prophylactically administer penicillin on the morning of and evening following dialysis, but we have not systematically investigated the need for this. Except for an occasional suture abscess at the cut-down site, we have not seen infection resulting from the procedure.

DISCUSSION

That a mechanically simpler, more compact, equally safe and efficient hemodialyzer will eventually be available seems likely. Currently, however, efforts in this laboratory and elsewhere to devise such an apparatus have fallen short of success through failure to appreciate the structure of the dialyzing membrane, the principles of diffusion through semipermeable membranes, and the hydraulic principles of flow across the surface of the membrane. Dialyzing efficiency is dependent not only on the ratio of the membrane area to which a given volume of blood is exposed, but also on membrane thickness, total pore area per unit area of membrane and, for larger molecules, on the average radius of the pores of the membrane. It is also most significantly dependent upon film resistance resulting from the laminar flow of bath liquid and the more viscous blood adjacent to the membrane. In the apparatus originally devised by Kolff, the factor of the film resistance is ingeniously minimized by the rapidly rotating drum to an extent not yet achieved by mechanically simpler stationary hemodialyzers.

In attempting to reduce the size of the apparatus for portability and bedside use, one must recognize, however, that the procedure is by and large an elective one, seldom being required on such short notice as a few hours. As

*Wolf and associates[6] has shown that the volume of blood in the Cellophane loops varies with blood flow through the apparatus.

such this procedure deserves its own special room. Considering the surgical equipment and space required for arterial and venous cannulation, carbon dioxide cylinders, cellophane boiler, sink and storage space for chemicals and sterilized equipment, it is obvious that, at least for a hemodialyzer built on the Kolff principle, reducing the size of the unit eventually reaches the point of the tail wagging the dog.

The mechanical ability required to assemble and operate this apparatus clinically is not great and can be acquired in a few weeks. The knowledge of cardiorenal disease required to decide when the procedure can be usefully applied is, of course, far less readily acquired.

The authors wish to express their gratitude to Dr. David Hume and members of the surgical house staff for their skill and care in performing the cannulations, and to Dr. John Pappenheimer, Department of Physiology, Harvard Medical School, Mr. George Jernstedt, Westinghouse Electric Corporation, and Dr. Gilbert Monet, Du Pont Company for indicating to us some of the factors in dialysis herein discussed.

SUMMARY

Technical improvements contributing to the safety, effectiveness, and simplicity of clinical hemodialysis are described. These include the substitution of autoclavable hemorepellent plastics, a technique for making satisfactory arterial and venous cannulae, redesign of the rotating coupling and venous return pump, reduction in the number of parts and connections and the time required for assembly, changes in bath composition and a more satisfactory concentration of bath pH. Principles of clinical hemodialysis and extracorporeal blood circulation are discussed.

Materials and equipment referred to herein may be obtained from the following sources: polyvinyl tubing, Irvington Varnish and Insulation Co., New Jersey; Kel-F, Plax Corporation, Hartford, Conn., Cellophane, Visking Corporation, Chicago; cyclohexanone and plastic clot and bubble trap, Fenwall Laboratories, Inc., Framingham, Mass. The Hemodialyzer is fabricated by Edward A. Olson, Ashland, Mass.

REFERENCES

1. Merrill, J. P., Thorn, G. W., Callahan, E. J., III, and Smith, L. H., Jr.: The Use of an Artificial Kidney: I. Technique, J. Clin. Investigation **29**: 412-424, 1950.
2. Merrill, J. P., Smith, S., III, Callahan, E. J., III, and Thorn, G. W.: The Use of an Artificial Kidney: II. Clinical Aspects, J. Clin. Investigation **29**: 425-438, 1950.
3. Merrill, J. P.: Present Role of the Artificial Kidney in Clinical Therapy, Ann. Int. Med. **33**: 100-107, 1950.
4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry: II. Methods, The Williams and Wilkins Co., p. 547-551, Baltimore, 1932.
5. Hastings, A. B., and Sendroy, J.: Studies of Acidosis, the Colorimetric Determinations of Blood pH at Body Temperature Without Buffer Standards, J. Biol. Chem. **61**: 695-710, 1924.
6. Wolf, A. V., Remp, D. G., Kiley, J. E., and Currie, G. D.: Artificial Kidney Function: Kinetics of Hemodialysis, J. Clin. Investigation **30**: 1062-1070, 1951.

USE OF THE WHOLE BLOOD OXIMETER DURING CARDIAC CATHETERIZATION

HERBERT S. HARNED, JR., M.D.,* PAUL R. LURIE, M.D.,**
CHARLES H. CROTHERS, M.D., AND RUTH WHITTEMORE, M.D.
NEW HAVEN, CONN.

THE purpose of this study was to evaluate the accuracy of the cuvette or whole-blood oximeter in measuring absolute oxygen saturation values and to evaluate the oximeter as an aid to the operator at the time of cardiac catheterization. The instrument used was a commercially produced cuvette oximeter similar to that developed by Groom and Wood.[1]

Karl Matthes,[2, 3] in 1935, was the first to develop the use of photoelectrimetry for measuring the oxygen saturation of blood and to develop the oximeter, an instrument which could be applied to measurements of arterial blood in the human ear. Kramer[4, 5] concurrently was studying spectral transmission characteristics of human blood and had shown that Beer's law of optical absorption applied to hemoglobin in unlaked red blood cells. Kramer developed an instrument that measured transmitted visible red light of 620-770 millimicrons, where oxyhemoglobin transmission is far better than reduced hemoglobin, but his instrument did not compensate for variations in hemoglobin concentration. Matthes' oximeter by using simultaneous red and green light absorption—where the red light absorption was a function of oxyhemoglobin and the green light absorption a function of total hemoglobin—was capable of measuring the ratio of oxyhemoglobin to total hemoglobin and thus oxyhemoglobin concentration.

Oximeters have been modified further by Matthes and Gross,[3] Goldie[6] and Squire[7] in England, Hartman[8] and McClure,[9] Millikan,[10] and Wood and associates[11-19] in the United States. Various absorption ranges have been used, Squire employing visible red and near infrared, Goldie visible red and blue, and Millikan and Hartman visible red and green. It is Wood's belief[17] that since the ear absorbs nearly all light of wave lengths shorter than 600 millimicrons and since the gelatin filters used by the investigators uniformly transmit near infrared light, Goldie and Millikan were recording near infrared light during earpiece oximetry instead of the blue or green as they had supposed.

A modification of the Millikan earpiece oximeter[10] was widely used in this country during World War II. This instrument required adjustment to a known value of arterial saturation (usually 98 to 100 per cent with the subject breathing air or 100 per cent oxygen), while the earpiece was in place in order to correct for the amount of ear tissue and blood in the optical path. Changes

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*James Hudson Brown Research Fellow in Pediatrics, Yale University School of Medicine. Part of this work was done during the tenure of a National Heart Institute Traineeship.

**Present address—Department of Pediatrics, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Ind.

in the amount of blood in the optical path were then compensated by bucking the output of the infrared cell against that of the red photocell. The Hartman oxyhemograph[8] operates similarly, except that the photocurrents are amplified, thus avoiding the use of the very sensitive galvanometers of the d'Arsonval type used in the Millikan oximeter.[20]

The accuracies reported by various investigators suggest that the Millikan earpiece oximeter shows a standard deviation of the differences between oximeter and Van Slyke values of 4 to 4.2 per cent. The Hartman instrument has been found to register within 2.5 per cent of Van Slyke values in 93 per cent of dual samplings in the high saturation ranges. As the result of an exhaustive study of the use of the earpiece oximeter, Wood[13, 14, 16] developed a technique for measuring absolute arterial oxygen saturations. He reported a standard deviation of the difference between oximetry readings and simultaneous Van Slyke[21] oxygen saturation determinations of 2.9 per cent in the saturation range of 13 to 100 per cent.

The earpiece oximeter has had wide applications to high altitude physiology and to aviation medicine as in human centrifugation experiments. It has been proved a useful instrument for study of arterial oxygen saturation during surgical anesthesia.[9, 22] The instrument has been used effectively in physiological studies of patients with congenital heart disease,[23-28] and has been used for determinations of circulation times.[29-32] The oximeter has been used for study of cases with bulbar poliomyelitis and for evaluation of the efficacy of respirator treatment of severe poliomyelitis cases.[33] Oximetry has been a valuable aid in the performance of the hypoxia test for evaluation of patients with coronary artery disease and of patients with various types of congenital heart disease.[34, 35] Methods for determining cardiac output are also being developed where the earpiece oximeter is employed to measure an estimation of "mixed venous blood" as well as a value for arterial oxygen saturation.[36, 37] Studies of the oxygen saturation of arterial blood of newborn infants by earpiece oximetry have been performed.[38]

METHOD

During diagnostic cardiac catheterization of thirty-eight children, ranging in age from 3 months to 15 years, whole blood oximetry was performed immediately prior to sampling for chemical analysis. The maximal time lapse between the two methods never exceeded twenty seconds.

All subjects were in a fasting state and most children below 8 years of age had received sodium pentothal per rectum and intravenously. In the experience of the authors, a "steady" state is well maintained by these measures.

Technique of Cuvette Oximetry Determinations and Calculations.—The oximeter* used in the study consisted of a "cuvette" cell, two galvanometers, a control box containing switches and potentiometers, a storage battery, and extension cables and plugs. In the cuvette cell, there was a multiple incandescent light source, a polyethylene tube with an internal volume of 0.5 ml. compressed to an ellipsoid with lumen depth of 0.58 mm. by a brass masking plate, and selenium barrier layer type photoelectric cells covered by appropriate gelatin filters. The polyethylene tube presented a proximal segment for attachment to the end of the catheter and a distal segment through which negative pressure was applied by syringe to

*The oximeter employed was manufactured commercially by the Waters-Conley Company of Rochester, Minn.

move the column of blood from the catheter through the cuvette. With this instrument, transmitted light was registered simultaneously in two different spectral regions, near infrared (750-900 millimicrons) and red (600-750 millimicrons).

This cuvette was calibrated before the present study in accordance with the method of Dr. Earl H. Wood.[17] The double-scale galvanometer had 100 equal scale divisions for both red and infrared. The two cell circuits were set to read 100 with saline flowing through the cuvette (Ro and IRo). The red and infrared deflections were recorded during slow flow of

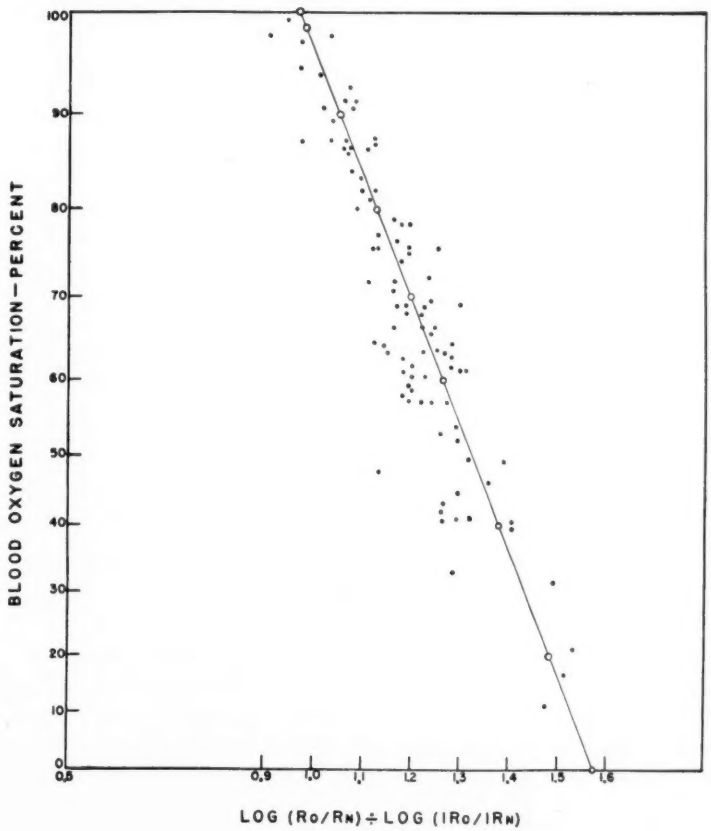

Fig. 1.

blood through the cuvette (Rn and IRn). A sample for chemical analysis was taken within twenty seconds. A calibration curve was made by plotting chemical determinations of percentage saturation against simultaneously determined oximetric "log ratio" values derived as follows:

$$(1) \quad \text{log ratio} = \log (Ro/Rn) \div \log (IRo/IRn)$$

This calibration curve for 100 determinations is shown in Fig. 1.

A nomogram (Fig. 2) was then established from this calibration curve to permit more convenient direct reading of percentage saturation from subsequently observed Rn and IRn readings. A series of integers spaced through the empirically occurring range of IRn (ten through twenty-one for this cuvette) was assumed. For each such number the Rn reading corresponding to each of a series of conveniently spaced percentage saturation levels could be calculated. This resulted in a series of curves for each IRn plotted against percentage

saturation on the ordinate and Rn logarithmically on the abscissa. The Rn reading for each IRn reading was computed as follows: IRn was assumed; IRo was 100; IRo/IRn was calculated; its log was taken. Transposing equation (1) gives:

$$(2) \quad \log (Ro/Rn) = \log \text{ ratio} \times \log (IRo/IRn)$$

Log ratio for the assumed percentage saturation was read off the calibration curve. Log (Ro/Rn) was then available by multiplication as in (2). Its antilog, Ro/Rn, was found. Ro being 100, Rn was found by dividing Ro/Rn into 100.

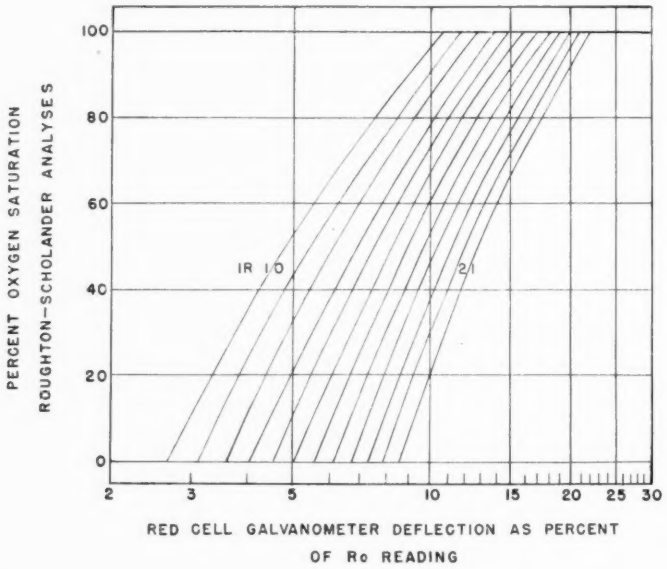

Fig. 2.

Technique for Chemical Analysis.—Samples for chemical analysis were collected in 2 ml. all-glass syringes which had been previously lubricated with mineral oil and moistened with sufficient 10 per cent potassium oxalate solution to fill the dead space. Immediately after the cuvette oximeter readings had been made, the springs were attached directly to the end of the catheter and blood samples withdrawn. After it was ascertained that no air bubbles were present, the blood samples were sealed under mercury and were placed in an ice and water bath. All samples were removed from the ice water bath immediately before analysis, which was done in all instances within six hours of sampling. Duplicate determinations of the oxygen content were carried out on 0.04 ml. aliquots of each sample by a modification of the Roughton-Scholander technique.[39-41] The analyses were carried out simultaneously by two technicians, and the determinations were considered satisfactory when they checked within 0.3 volume per cent before reduction to standard temperature and pressure conditions. Oxygen capacities were determined by transferring a 0.9 ml. aliquot of blood from representative samples during the procedure to a 20 ml. bottle and equilibrating with room air for thirty minutes on a mechanical rotator, before analysis by the microgasometric technique.

Calculations.—Values for oxygen content and capacities were expressed in volumes per 100 ml. of blood under standard conditions of temperature and barometric pressure. From the observed total oxygen capacity, the amount of oxygen carried in physical solution under conditions in the Roughton-Scholander tonometer was subtracted. Percentage oxygen saturations were calculated by the formula:

$$O_2 \text{ saturation } (\%) = 100 \times \frac{\text{Total } O_2 \text{ content} - \text{dissolved } O_2}{\text{Corrected } O_2 \text{ capacity}}$$

The values for dissolved oxygen were taken from data published elsewhere.[39, 42]

The accuracy of 0.15 to 0.20 volume per cent for gasometric oxygen analyses reported by Roughton and Scholander is of the same order as has been found in our laboratory. In lower saturation ranges the error by the modified Roughton-Scholander method has been larger than in high saturation ranges. It is our impression that this microgasometric method has many technical advantages over the Van Slyke method while preserving a high degree of accuracy.

RESULTS

Accuracy of the Instrument.—Thirty-eight patients were studied and 190 cuvette oximetry readings were available for comparison with simultaneous gasometric analyses. The over-all standard deviation of the difference between the two determinations was 4.2 per cent. This error was influenced markedly by poor agreement of the methods below gasometric saturations of 70 per cent. The standard deviation was 3.6 per cent in the range of gasometric saturations of 70 to 100 per cent, which included 145 of the 190 comparisons. The standard errors would appear excessive for the use of the cuvette oximeter values under the conditions of the study as substitutes for the gasometric analyses or for consideration of the values obtained as absolute determinations.

Inaccuracy in the lower saturation ranges has been repeatedly noted in studies of earpiece oximetry,[17, 27, 28] but was not noted by Groom[1] in his study of the whole blood oximeter where an over-all standard deviation of the differences between oximetric and Van Slyke determinations of only 1.8 per cent oxygen saturation was observed. A wide scatter of values in the lower saturation ranges was observed by us as shown by the calibration curve (Fig. 1). No significant improvement in accuracy resulted from use of curves other than that illustrated. Although it has been demonstrated that the use of a polychromatic light source in the oximeter, Beer's law cannot be applied accurately to blood solutions, it would seem unlikely that this would explain the large scatter in the lower saturation ranges observed.

Possible errors in the study as it was constituted include: (1) errors in reading the oximeter, (2) errors in reading the nomogram, (3) changes in voltage supplied to the light source between samplings, (4) variations in the rate of flow through the cuvette cell,* (5) variations in the volume of blood between the light sources and photocells, (6) variations in the patient's "steady state" not suspected by the operator, and (7) minor changes in the position of the catheter. All of these factors would, of course, be operable in the high saturation ranges as well as the low. The greater error in gasometric analysis of samples of unsaturated blood may account in part for the larger differences between the two techniques in the lower saturation ranges.

Clinical Diagnostic Applications.—Despite the inaccuracies discussed above, the cuvette oximeter has proved very useful during diagnostic cardiac catheterization.

(a) *Representative Sampling.*—It is most important during cardiac catheterization that representative samples be taken from the sites selected. These samples should approximate as closely as possible the average mixed blood saturation of the chamber or vessel sampled, and such factors as proximity to

*Wood[17] has shown that calibration curves with blood stationary in the cuvette cell differ significantly from those with slow flow through the cuvette.

a source of highly unoxygenated or highly oxygenated blood and laminar flow in chambers should be considered. For example, a representative sample of right auricular blood should be taken from mid right auricle and not in the stream of blood from the coronary sinus, superior vena cava, inferior vena cava, or a left to right shunt through an atrial septal defect. The cuvette oximeter allows multiple sampling in a chamber with "on the spot" evaluation of the location of the catheter tip by fluoroscopy so that a representative sample may be obtained. By this technique, x-rays for location of the catheter tip and gasometric determinations of multiple samples from each chamber may be reduced in number.

(b) *Presumptive Diagnosis During Cardiac Catheterization.*—Determination of arteriovenous and venoarterial shunts has been one of the most important aspects of diagnostic cardiac catheterization. If an arteriovenous shunt is present, oxygen saturation values at the site of the shunt and at points distal to the shunt are higher than those at proximal sites. For example, in a case of interventricular septal defect, higher oxygen saturations will be observed in the right ventricle—where the stream of highly saturated blood from the left ventricle enters—than in the right auricle and superior vena cava. If a significant venoarterial shunt is present, peripheral arterial samples will be unsaturated. The whole blood oximeter has proved successful in providing a presumptive diagnosis of arteriovenous shunt at the time of cardiac catheterization.

Table I presents the diagnoses made after a review of the cardiac catheterizations of the thirty-eight patients. For diagnosis of patent ductus arteriosus a one volume per cent increment in gasometrically determined oxygen content between distal right ventricle and pulmonary artery was considered necessary and for diagnosis of interventricular septal defect, a one volume per cent increase in oxygen content in a right ventricular sample over proximal samples was accepted. Diagnosis of interauricular septal defect was made when the oxygen content of the right auricle exceeded the averaged vena caval oxygen content by two volumes per cent.

Eighteen cases of arteriovenous shunt satisfied the above criteria for diagnosis. In seventeen of these cases, cuvette oximetry saturations were available for comparison with gasometrically determined saturations at points proximal and distal to the shunt. In Table II, the increment (Dg) of oxygen saturation between the sample drawn proximal to the shunt and that drawn distal to it as analyzed gasometrically is recorded for each patient, and this is compared with the increment (Dc) between the same sites determined by cuvette oximetry. Listed also are the differences of the cuvette increments from the gasometric increments (Dc–Dg) expressed as per cent oxygen saturation. Presumptive diagnoses from cuvette oximetry are compared with the diagnoses after gasometric samples were reviewed.

In eleven of the seventeen cases, cuvette increments of 8.0 per cent oxygen saturation or greater were observed between the sites proximal and distal to the

TABLE I. DIAGNOSES BY CARDIAC CATHETERIZATION STUDY IN 38 PATIENTS

DIAGNOSIS BY CARDIAC CATHETERIZATION	NO. CASES	PATIENTS' INITIALS
Interventricular septal defect	5	A. B., B. C., R. C., J. H., P. V.
Interventricular septal defect by passage of catheter	1	S. M.
Eisenmenger's complex	3	G. B., D. G., D. G.
Interventricular septal defect plus patent ductus arteriosus	1	D. B.
Patent ductus arteriosus	3	P. C., J. R., J. W.
? Patent ductus arteriosus	1	M. E. T.
Patent ductus arteriosus plus coarctation of the aorta	1	M. B.
Patent ductus arteriosus (no cuvette samples obtained at site of arteriovenous shunt)	1	G. B.
Interauricular septal defect	2	J. C., C. G.
Interauricular septal defect by catheter passage not demonstrated by cuvette or gasometric saturations	1	M. J. C.
? Interauricular septal defect	1	E. M.
Interauricular septal defect plus interventricular septal defect	2	B. G., J. L.
Interauricular septal defect plus valvular pulmonic stenosis	1	F. C.
Interauricular septal defect plus anomalous right pulmonary vein emptying into right auricle plus other abnormalities	1	M. K.
Pulmonic stenosis (valvular)	1	J. M.
Tetralogy of Fallot plus interauricular septal defect	2	A. S., J. S.
No diagnosis made by cardiac catheterization alone	11	J. B., R. C., R. C., S. C., P. D., R. K., L. R., E. S., C. S., J. T., R. Z.

shunt. In four cases increments of 6.0 to 8.0 per cent oxygen saturation were noted, and in two cases increments of only 2.0 and 4.0 per cent oxygen saturation were observed.

From the data from all thirty-eight cases, the largest increment in oxygen saturation observed by cuvette oximetry where no significant increment was noted by gasometric analysis was 6.0 per cent, and no other increments greater than 4.5 per cent oxygen saturation were observed. The patient showing the increment of 6.0 per cent oxygen saturation by cuvette oximetry showed a gasometric increment of 3.7 per cent oxygen saturation (0.67 volume per cent oxygen content) between the right ventricular outflow tract and the pulmonary artery. This child (A. B. in Table II) was in congestive failure at the time of cardiac catheterization and developed a cardiac arrhythmia. Both methods revealed a high ventricular septal defect with increments of 16.5 per cent oxygen saturation by cuvette oximetry and 16.4 per cent by gasometric recordings between mid right ventricle and right ventricular outflow tract.

Two other cases are presented where cuvette oximetry challenged the diagnosis by gasometric analysis. In the first case, E. M., an increment of 15.0 per cent oxygen saturation was observed between inferior vena caval and right auricular cuvette samples compared to an increment of 10.1 per cent oxygen saturation and 1.6 volume per cent oxygen content by gasometric analysis. In this case, the catheter was readily passed into the left auricle directly from the right auricle. Presumptive diagnosis of interauricular septal defect by cuvette

TABLE II. COMPARISON OF CUVETTE INCREMENT AND GASOMETRIC INCREMENT IN PER CENT OXYGEN SATURATION WITH CORRESPONDING DIAGNOSES

PATIENT	CUVETTE INCREMENT (Dc) AND SITES OF SAMPLES (PER CENT)	GASOMETRIC INCREMENT (Dg) (PER CENT)	DIFFERENCE BETWEEN Dc AND Dg (PER CENT)	DIAGNOSES	
				CUVETTE	GASOMETRIC
D. B.	7.0 (RV-RA)	9.0	−2.0	IVSD*	IVSD*
A. B.	16.5 (RV outflow-RV mid)	16.4	+0.1	High IVSD	High IVSD
	6.0 (MPA-RV outflow)	3.7	+2.3	? PDA	----
M. B.	14.5 (MPA-RV outflow)	13.9	+0.6	PDA	PDA† ‖
G. B.	20.0 (RV-RA)	17.6	+2.4	IVSD	IVSD‡
J. C.	19.5 (RA-SVC)	19.6	−0.1	IASD	IASD
B. C.	38.0 (RV-RA)	43.2	−5.2	IVSD	IVSD
P. C.	8.0 (MPA-RV)	10.7	−2.7	PDA	PDA† ‖
R. C.	14.0 (RV-RA)	20.3	−6.3	IVSD	IVSD
C. G.	11.5 (RA-SVC)	14.5	−3.0	IASD	IASD
D. G.	2.0 (RV outflow-RV apex)	5.9	−3.9	----	IVSD‡
D. G.	18.5 (mid RV-RA)	20.6	−2.1	IVSD	IVSD‡
B. G.	7.5 (mid RV-RA)	8.2	−0.7	IVSD	IVSD & IASD§
J. H.	20.0 (RV-RA)	20.2	−0.2	IVSD	IVSD
J. L.	4.0 (RV-RA)	7.3	−3.3	----	IVSD & IASD§
E. M.	15.0 (RA-IVC)	10.1	+4.9	IASD	? IASD
J. R.	6.0 (MPA-RV outflow)	8.6	−2.6	PDA	PDA‖
M. E. T.	4.5 (MPA-RV outflow)	9.0	−4.5	----	PDA (?)
P. V.	12.0 (RV-RA)	8.7	+3.3	IVSD	IVSD
J. W.	7.0 (MPA-RV outflow)	12.6	−5.6	PDA	PDA‖

MPA—Main pulmonary artery; RV—right ventricle; RA—right auricle; SVC—superior vena cava; IVC—inferior vena cava.

*Plus PDA by passage of catheter into aorta through ductus (proved by operation).

†Diagnosis of coarctation of aorta made by direct arterial pressures.

‡Diagnosis of Eisenmenger's complex made on basis of arterial unsaturation.

§No cuvette SVC samples taken for demonstration of IASD.

‖Proved at operation by Dr. W. L. Glenn.

oximetry was supported by physical examination and roentgenologic findings. A second case, M. E. T., revealed an increment of only 4.5 per cent oxygen saturation by cuvette oximetry between right ventricular outflow and main pulmonary artery samples compared to an increment of 9.0 per cent oxygen saturation (1.6 volumes per cent oxygen content) by gasometric analysis. Absence of a continuous murmur in this 8-year-old girl casts doubt on the diagnosis of patent ductus arteriosus made by gasometric analysis.

Therefore, of seventeen cases where a diagnosis of arteriovenous shunt was made by gasometric analysis using the criteria specified, fifteen had revealed presumptive evidence of shunt by cuvette oximetry at the time of cardiac catheterization. In one case, cuvette oximetry revealed an arteriovenous shunt, not confirmed by gasometric analysis, but probably correctly located, and in another case cuvette oximetry did not reveal an arteriovenous shunt which was probably fictitiously localized by gasometric sampling. One case showed probable fictitious evidence of an arteriovenous shunt by cuvette oximetry which was not confirmed by gasometric analysis.

None of the other cases in the group of thirty-eight patients presented significant increments for comparison. Femoral artery samples were taken by direct puncture on all patients, but because of technical difficulties inherent in this procedure, no samples of femoral artery blood were taken for cuvette analysis. The effectiveness of the whole blood oximeter in determining the presence of venoarterial shunts was not evaluated in the present study.

Under the conditions of the study, the cuvette oximeter measured increments in oxygen saturation more accurately than it measured absolute oxygen saturations. In the above discussion, rigid criteria were used for the diagnosis of an arteriovenous shunt. It has been shown[43] that these concepts of a significant increment necessary to demonstrate an arteriovenous shunt are too rigid in view of the considerable inherent variability of right heart samples during diagnostic cardiac catheterizations, even when uninfluenced by a shunt. Thus, a series of samples showing repetition of an increment is more significant than a single pair. The cuvette oximeter, by giving instantaneous indication of an increment in saturation of borderline significance, can prompt the operator to take a series of samples about the point in question. Likewise, if increments can be repeatedly demonstrated by multiple cuvette sampling proximal and distal to an arteriovenous shunt, the diagnosis may be established far better than by single proximal and distal gasometric samples. The ease of determining oxygen saturation of the blood by cuvette oximeter in contrast to the laboriousness of gasometric technique makes multiple sampling by the cuvette feasible in such instances.

The presumptive diagnostic evidence gained at the time of cardiac catheterization by use of the whole blood oximeter was of value in decreasing the time of the procedure and of great value in aiding the operator to adapt the procedure to meet the individual diagnostic problem.

(c) *Unusual Locations of the Catheter Tip.*—Not infrequently during cardiac catheterization, the catheter tip was passed into an unusual location which was not readily discerned by the operator. Under certain circumstances, pressure tracings and fluoroscopic examination with the catheter tip in these unusual sites were not diagnostic for the chamber or vessel involved. During the cardiac catheterizations included in this study, unusual locations entered included (1) the hepatic vein or renal vein from the inferior vena cava, (2) the coronary sinus via the right auricle, (3) the coronary sinus via a persistent left superior vena cava, (4) the right auricle via a persistent left superior vena cava and coronary sinus, (5) the left pulmonary vein via an interauricular septal defect and the left auricle, (6) the right pulmonary vein (aberrant) from the right auricle, (7) the aorta via an interventricular septal defect, (8) the aorta via a patent ductus arteriosus, and (9) the distal pulmonary artery with sampling of pulmonary capillary blood. All of these locations have been reported entered by others, often by design. The cuvette oximeter has aided in the immediate identification of these unusual locations, although at times confirmatory pressures need be taken. Time formerly spent in recording pressures, taking x-rays, and performing chemical analyses in order to locate the tip of the catheter has been saved.

(d) *Evaluation of "Steady" State During Catheterization.*—On certain occasions, changes in the patient's basal state and circulatory dynamics, at times of alarming nature, were detected by the cuvette oximeter. On several occasions, in small children with pulmonic stenosis, a lowered saturation was noted as the catheter was placed in the pulmonary artery. If this saturation was strikingly lower than a sample previously obtained from the right ventricle, the catheter was assumed to be significantly occluding the pulmonary outflow and it was

withdrawn immediately. Also, mild cardiac irregularities not severe enough to cause termination of the procedure without other evidence of cardiac embarrassment, were at times accompanied by significant drops in saturation by cuvette oximetry, which warned the operator of impending danger.

(e) *Cardiodynamic Studies During Cardiac Catheterization.*—From the present study, it became apparent that the cuvette oximeter might be widely used for studying different aspects of cardiac physiology. Oxygen saturation measurement during the use of volatile anesthetic agents which interfere with usual gasometric techniques, studies of the pathological changes in congenital cardiac patients subjected to various forms of stress, and the use of the cuvette oximeter during hypoxia tests represent suggested applications. Burchell and associates[23, 25] have used the cuvette oximeter and earpiece oximeter for simultaneous determinations of arterial and mixed venous blood saturations in patients with cyanotic congenital heart disease.

SUMMARY

The use of the whole blood or cuvette oximeter during cardiac catheterization was studied by employing a commercially produced oximeter similar to that developed by Groom and Wood.

Comparisons of 190 cuvette oximeter recordings from thirty-eight cases of suspected congenital heart disease were made with samples analyzed by a modification of the Roughton-Scholander microgasometric method for blood oxygen saturation.

Under the conditions of the study, the over-all standard deviation of the difference between the two methods of 4.2 per cent and the standard deviation of 3.6 per cent for the gasometric saturation range of 70 to 100 per cent would appear excessive for the use of the cuvette oximeter values as substitutes for the gasometric analyses or for consideration of the values obtained as absolute readings.

The use of the whole blood oximeter at the time of cardiac catheterization provides information of value pertaining to (1) immediate presumptive diagnosis, (2) sampling from representative sites, (3) unusual locations of the catheter tip, and (4) basal state of the patient.

Under the conditions of the study, more accuracy was obtained in determining increments between blood oxygen saturations recorded successively by the cuvette oximeter than in determining absolute values. Of seventeen cases where a diagnosis of arteriovenous shunt was made by gasometric analyses, fifteen had given strong evidence by oximetry of shunts such as those later diagnosed. Only one case showed presumptive evidence of an arteriovenous shunt by oximetry who did not show evidence of a similar shunt by gasometric analyses.

Cuvette oximetry was a most useful adjunct to the technique of diagnostic cardiac catheterization.

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REFERENCES

1. Groom, Dale, Wood, Earl H., Burchell, Howard B., and Parker, Robert L.: The Application of an Oximeter for Whole Blood to Diagnostic Cardiac Catheterization, Proc. Staff Meet., Mayo Clin. **23**: 601, 1948.
2a. Matthes, K.: Über den Einfluss der Atmung auf die Sauerstoffsättigung des Arterienblutes, Arch. f. exper. Path. u. Pharmakol. **176**: 683, 1934.
b. Matthes, K.: Untersuchungen über die Sauerstoffsättigung des menschlichen Arterienblutes, Arch. f. exper. Path. u. Pharmakol. **179**: 698, 1935.
c. Matthes, K.: Untersuchungen über den Gasaustausch in der menschlichen Lunge. Sauerstoffgehalte und Sauerstoffspannung im Arterienblut und im venösen Mischblut des Menschen, Arch. f. exper. Path. u. Pharmakol. **181**: 630, 1936.
d. Matthes, K.: Untersuchungen über den Gasaustausch in der menschlichen Lunge: der Ausgleich der Sauerstoffspannung zwischen Alveolen und Blute, Arch. f. exper. Path. u. Pharmakol. **181**: 640, 1936.
3a. Matthes, K., and Gross, F.: Untersuchungen über die Absorption von rotem und ultrarotem Lichte durch kohlenoxydgesättigtes, sauerstoffgesättigtes und reduziertes Blut., Arch. f. exper. Path. u. Pharmakol. **191**: 369, 1938.
b. Matthes, K., and Gross, F.: Fortlaufende Registrierung der Lichtabsorption des Blutes in zwei verschiedenen Spektralbezirken, Arch. f. exper. Path. u. Pharmakol. **191**: 381, 1939.
c. Matthes, K., and Gross, F.: Fortlaufende Registrierung von Kohlenoxydhämoglobin im strömenden Blute, Arch. f. exper. Path. u. Pharmakol. **191**: 319, 1939.
d. Matthes, K., and Gross, F.: Zur Method der fortlaufenden Registrierung der Farbe des Menschlichen Blutes, Arch. f. exper. Path. u. Pharmakol. **191**: 523, 1939.
e. Matthes, K., and Gross, F.: Über den Nachweis von Methämoglobin und Cyanmethämoglobin im Strömenden Blute, Arch. f. exper. Path. u. Pharmakol. **191**: 706, 1939.
4. Kramer, K.: Bestimmung des Sauerstoffgehaltes und der Hämoglobinkonzentration in Hämoglobinlösungen und hämolysiertem Blut auf licktelektrischem Wege, Zeitschr. f. Biol. **95**: 126, 1934.
5. Kramer, K.: Ein Verfahren zur fortlaufenden Messung des Sauerstoffgehaltes im strömenden Blute an uneröffneten Gefässen, Zeitschr. f. Biol. **96**: 61, 1935.
6. Goldie, E. A. G.: Device for Continuous Indication of Oxygen Saturation of Circulating Blood in Man, Rev. Scient. Instruments **19**: 23, 1942.
7. Squire, J. R.: Instrument for Measuring Quantity of Blood and Its Degree of Oxygenation in Web of the Hand, Clinical Science **4**: 331-337, 1940.
8. Hartman, F. W., Behrman, Vivian G., and Chapman, F. W.: Photo-electric Oxyhemograph: Continuous Method for Measuring Oxygen Saturation of Blood, Am. J. Clin. Path. **18**: 1, 1948.
9. McClure, Roy D., Behrmann, Vivian G., and Hartman, Frank W.: The Control of Anoxemia During Surgical Anesthesia with Aid of an Oxyhemograph, Ann. Surg. **128**: 685, 1948.
10. Millikan, Glenn A.: Oximeter—An Instrument for Measuring Continuously Oxygen Saturation of Arterial Blood in Man, Rev. Scient. Instruments **13**: 434, 1942.
11. Wood, Earl H., Geraci, Joseph E., Pollack, Albert, Groom, Dale, Taylor, Bowen, Pender, John W., and Pugh, David G.: General and Special Techniques in Cardiac Catheterization, Proc. Staff Meet., Mayo Clin. **23**: 494, 1948.
12. Wood, Earl H., Geraci, Joseph E., and Groom, Dale: Photoelectric Determination of Blood Oxygen Saturation in Man, Fed. Proc. **7** (part I): 137, 1948.
13. Wood, Earl H.: Normal Oxygen Saturation of Arterial Blood During Inhalation of Air and Oxygen, J. Appl. Physiol. **1**: 567, 1949.
14. Wood, Earl H., and Geraci, J. E.: Photoelectric Determination of Arterial Oxygen Saturation in Man, J. Lab. & Clin. Med. **34**: 387, 1949.
15. Wood, Earl H.: A Single Scale Absolute Reading Oximeter, J. Lab. & Clin. Med. **34**: 1767, 1949.
16. Wood, Earl H., Knutson, J. R. B., and Taylor, B. E.: A Comparison of Oximetric Measurements on Histaminized and Heat-Flushed Ears, Am. J. Physiol. **159**: 597, 1949.
17. Wood, Earl H.: Oximetry. Medical Physics (Year Book Publishers), Vol. II, p. 664, 1950.
18. Comroe, J. H., Jr., and Wood, Earl H.: Ear Oximeters. Methods in Medical Research (Year Book Publishers), Vol. II, p. 145, 1950.
19. Comroe, J. H., Jr., and Wood, Earl H.: Channel Oximeters. Methods in Medical Research (Year Book Publishers), Vol. II, p. 154, 1950.
20. Liston, Max D., Quinn, C. E., Sargeant, W. E., and Scott, C. G.: A Contact Modulated Amplifier to Replace Sensitive Suspension Galvanometers. Rev. Scient. Instruments **17**: 194, 1946.

21. Van Slyke, D. D., and Neill, J. M.: Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, I, J. Biol. Chem. **61**: 523, 1924.
22. Faulconer, A., Pender, J. W., and Bickford, R. G.: The Influence of Partial Pressures of Nitrous Oxide on the Depth of Anesthesia and the Electro-encephalogram in Man, Anesthesiology **10**: 601, 1949.
23. Burchell, Howard B., and Wood, Earl H.: Reproducibility of Values for Oxygen Saturation of Arterial Blood and Magnitude of Venous-Arterial Shunts in Patients with Congenital Cardiac Malformations, J. Appl. Physiol. **1**: 560, 1949.
24. Burchell, Howard B., and Wood, Earl H.: Remarks on the Technique and Diagnostic Applications of Cardiac Catheterization, Proc. Staff Meet., Mayo Clin. **25**: 41, 1950.
25. Burchell, Howard B., Taylor, Bowen E., Knutson, Julian R. B., and Wood, Earl H.: Circulatory Adjustments to the Hypoxemia of Congenital Heart Disease of the Cyanotic Type, Circulation **1**: 404, 1950.
26. Burchell, Howard B.: Introduction to Clinical Applications of Oximetry, Proc. Staff Meet., Mayo Clin. **25**: 377, 1950.
27. Gullickson, Glenn, Elam, J. O., Hammond, Margaret, Paine, J. R., and Varco, R. L.: Oxygenation Studies in Congenital Pulmonary Stenosis: An Application of Recording Oximetry in the Evaluation of Cardiorespiratory Function, Am. Heart J. **35**: 940, 1948.
28. Montgomery, G. E., Jr., Wood, E. H., Burchell, H. B., Dry, T. J., Parker, R. L., and Helmholz, H. F., Jr.: Continuous Observations of Arterial Oxygen Saturation at Rest and During Exercise in Congenital Heart Disease, Am. Heart J. **36**: 668, 1948.
29. Jablons, Benjamin, and Cohen, Jules: Clinical Studies of Circulation Time with Objective (Photo-electric Cell-Dye) Method, New York State J. Med. **44**: 398, 1944.
30. Knutson, Julian, Taylor, Bowen, Ellis, Eugene, and Wood, Earl H.: Studies on Circulation Time with the Aid of the Oximeter, Proc. Staff Meet., Mayo Clin. **25**: 405, 1950.
31. Miller, B. J.: Circulation Time: An Accurate Indicator for Objective Measurement, J. LAB. & CLIN. MED. **33**: 910, 1948.
32. Wexler, J., Whittenberger, J. L., and Himmelfarb, Sylvia: An Objective Method for Determining Circulation Time from Pulmonary to Systemic Capillaries by Use of the Oximeter, J. Clin. Investigation **25**: 447, 1946.
33. Elam, J. O., Hemingway, Allan, Gullickson, G., and Visscher, M. B.: Impairment of Pulmonary Function in Poliomyelitis: Oximetric Studies in Patients with Spinal or Bulbar Types, Arch. Int. Med. **81**: 649, 1948.
34. Penneys, Raymond, and Thomas, Caroline B.: Oximeter Control of Arterial Oxygen Saturation in Anoxemia Studies, Bull. Johns Hopkins Hosp. **82**: 470, 1948.
35. Penneys, Raymond, and Thomas, Caroline B.: The Relationship Between Arterial Oxygen Saturation and Cardiovascular Response to Induced Anoxemia in Normal Young Adults, Circulation **1**: 415, 1950.
36. Knutson, Julian, Taylor, Bowen, and Wood, Earl H.: Oximetric Method for Determination of the Oxygen Saturation of Mixed Venous Blood in Man, Fed. Proc. 8: 87, 1949.
37. Roos, Albert, Elam, J. O., Neville, J. F., and White, H. L.: Oximetric Determination of Cardiac Output in Man, Fed. Proc. **7**: 104, 1948.
38. Crehan, Elmer L., Kennedy, Roger L. J., and Wood, Earl H.: A Study of the Oxygen Saturation of Arterial Blood of Normal Newborn Infants by Means of a Modified Photo-electric Oximeter: Preliminary Report, Proc. Staff Meet., Mayo Clin. 25: 392, 1950.
39. Ordway, Nelson K.: To be published.
40. Preston, Sylvia N., and Ordway, Nelson K.: Observations of Arterial Oxygen Content in Children During the Inhalation of Air and 100% Oxygen, Am. J. Physiol. **152**: 696, 1948.
41. Roughton, F. J. W., and Scholander, P. F.: Microgasometric Estimation of Blood Gases, Oxygen, J. Biol. Chem. **148**: 541, 1943.
42. Sendroy, Julius, Jr., Dillon, R. T., and Van Slyke, D. D.: Studies of Gas and Electrolyte Equilibria in Blood. XIX. The Solubility and Physical State of Uncombined Oxygen in Blood, J. Biol. Chem. **105**: 597, 1934.
43. Lurie, P. R., Gray, F. D., Jr., and Whittemore, Ruth: Cardiac Catheterization and Other Physiological Studies in 50 Cases of Congenital Heart Disease, Angiology, **3**: 98, 1952.

THE CONVERSION OF MILLIKAN AND WOOD TYPE OXIMETERS INTO DIRECT-WRITING RECORDING INSTRUMENTS FOR USE IN SURGERY, IN STUDIES OF PULMONARY FUNCTION, AND IN TEACHING RESPIRATORY PHYSIOLOGY

JOHN F. PERKINS, JR., M.D., WILLIAM E. ADAMS, M.D., AND HUBERTA LIVINGSTONE, M.D.
CHICAGO, ILL.

WITH THE TECHNICAL ASSISTANCE OF RICHARD A. WEAVER, A.B., WILLIAM H. JOHNSON, B.S., AND S. H. BRYANT, B.S.

THIS article describes the relatively simple technical procedures whereby an individual with only moderate experience in electronics can assemble a Millikan or Wood type recording oximeter at minimal cost. Three of us, students respectively in Medicine (R. A. W.) and in Physiology (W. H. J. and S. H. B.), have constructed four such intruments, no two exactly alike. Two are of the Millikan, relative reading type and two are of the Wood, absolute reading type. This article is written in response to requests from visitors to the University of Chicago Clinics for details of construction of these recording oximeters. Neither a contribution to the basic theory of oximetry, nor a first account of recording oximeters (see "Brief Review of Principles" below), it is directed to those physicians and scientists from hospitals, medical schools, and universities who may wish to have a reliable recording oximeter assembled in their laboratories. To our knowledge, no detailed instructions for this purpose are now available.

The first of our recording oximeters was assembled in January, 1950, because it was believed it would be useful in treating a patient in whom it became necessary for one of us (W. E. A.) to undertake closure of several arteriovenous shunts in the patient's lung. The other lung had been removed seven years earlier for the same condition.[1] The resulting instrument contributed materially, we believe, to the successful management of the case,[2] not only at operation, but as a means of evaluating the pulmonary reserve of this patient before and after operation. In the ensuing two years the four recording oximeters assembled in our laboratory have been used in the University of Chicago Clinics on over 250 patients during surgery. In addition, they have been used in pre- and postoperative studies of pulmonary function on over 80 patients, in studies of pulmonary function on animals with experimentally produced pulmonary lesions, in measurement of cardiac output in infants and children by the dye method of Nicholson and associates,[3] in determination of saturation of blood with carbon monoxide by a new method,[4] and finally in

From the Departments of Physiology and Surgery, University of Chicago.
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laboratory exercises in courses in mammalian physiology. During this period, the recording oximeters have been found to posses certain advantages over the galvanometer type instruments, as follows:

(a) *Ruggedness:* The readings of the unit are virtually unaffected by the occasional jostling which any instrument may receive in the close quarters of the operating room. Moreover the instruments themselves are not readily damaged by vibration, as shown by the fact that two of them were recently transported 900 miles by automobile without special packing, and functioned satisfactorily upon arrival at their destination, and upon return.

(b) *Visibility:* By means of electronic amplification a pointer moves from 8 to 12 inches along a scale, permitting ready visibility on the part of the surgeon.

(c) *Continuous automatic recording* of the oxygen saturation has been obtained on permanent charts showing the complete time course of changes in oxygen saturation occurring during surgery of the heart, great vessels, and lungs, during the use of various types of anesthetics,[5] and during studies of pulmonary function in human beings and animals.[6]

(d) *Speed of response:* Two of these instruments have a period, or response time of approximately one second, representing a fraction only of the response time in the galvanometers regularly used with barrier cell oximeters. This rapid response has revealed, for example, marked shifts in oxygen saturation correlated with respiration in anesthetized dogs.

BRIEF REVIEW OF BASIC PRINCIPLES OF OXIMETRY

For a detailed description of the basic theory of oximetry, as developed over the past twenty years by Nicolai,[7] Millikan,[8-10] Kramer,[11] Matthes,[12] Matthes and Gross,[13] Brinkman and Wildschut,[14] Squire,[15] Hartman and McClure,[16] Goldie,[17] Wood and Geraci,[18] and Wood,[19, 20] Elam, Sleator and associates,[21, 22] and Paul,[23] the reader is referred to the review articles of Wood,[20] Comroe and Wood,[24] and Elam and associates,[21] as well as to the original articles cited. Only a brief description of the principles of oximetry will be included here.

Detecting Units, Millikan and Wood Oximeters.—The "heart" of these oximeters is (1) the oximeter earpiece, which fits over the pinna of the ear, and (2) the oximeter cuvette for whole blood. Each contains two selenium barrier-layer photocells, one receiving red light, the other infrared light. The blood, in the tissues of the ear or within the lumen of the cuvette, is interposed between a source of light and the photocells. In cyanosis the bluish tint of the blood absorbs red light in a relation proportional to the logarithm of the per cent saturation of the hemoglobin. As a result, the "red" photocell receives less light and therefore produces less voltage.

The photocell for infrared is utilized to compensate for the quantity of hemoglobin present in the ear or in the cuvette, and for the opacity of the tissue of the ear. This photocell is virtually insensitive to changes in oxygen saturation of the blood. By adding its electrical output algebraically to that of the photocell for red, Millikan,[10] obtained an instrument which, while responsive to changes in oxygen saturation, was largely unaffected by changes in the quantity of the blood present in the tissues of the ear. The *Millikan oximeter* has, in the ten years since its development, proved extremely valuable, having extensive use during World War II in the training of fliers who were to go to high altitudes, and subsequently during studies of pulmonary and cardiac function,[24] during anesthesia,[25, 26] and in teaching respiratory physiology. Two of our recording oximeters, while utilizing the Millikan earpiece, have been used in over 100 operations, in several of which they have proved invaluable. In spite of its excellent record, the Millikan oximeter has certain limitations.

It must first be set to the per cent saturation of the arterial blood (97 per cent in normal subjects breathing air, 100 per cent if breathing oxygen, Van Slyke analyses required in chronically cyanotic patients). In this sense, then, the Millikan oximeter is a "relative reading" instrument. Its initial settings may, moreover, gradually change or "drift" slightly.

The *Wood oximeter,* representing the first "absolute reading" oximeter, was described in 1949 and 1950 by Wood and Geraci,[18] and by Wood.[19] Its earpiece is similar to that of the Millikan oximeter, but there has been added a pressure capsule, arranged so as to render the ear bloodless while making the initial instrumental setting for a given subject. Wood's "two-scale" instrument requires simultaneous readings of galvanometers connected respectively to the "red" and "infrared" photocells. From these readings, the per cent saturation can be calculated or read from a nomogram once the instrument has been calibrated with the help of ingenious filters developed by Wood for the purpose, or by means of Van Slyke analyses. Wood's "single-scale" oximeter gives direct readings without calculations, though its accuracy (S. D. 2.6 per cent for saturations above 95 per cent, 5.2 for those below), is somewhat less than that of the two-scale instrument. Among the advantages of the Wood oximeters are: (1) they can be used to measure the oxygen saturation of chronically cyanotic patients whose blood does not reach 100 per cent even when breathing pure oxygen, (2) the instrument may be reset at any time, thereby minimizing errors due to drift.

Requirements of an Amplifying Unit for a Recording Oximeter.—The "difference" voltage from the two photocells of the Millikan or single scale Wood oximeter is of the order of 1 millivolt or less. While sufficient to swing a very sensitive galvanometer, this voltage is not large enough to operate a stable, direct coupled amplifier capable of driving a direct writing device.

Millikan[27] solved this problem in 1942 by using photoelectric amplification. In this technique, previously developed for other purposes, a beam of light, reflected from a mirror mounted on the moving coil of a galvanometer, comes to fall on various portions of adjacent photocells. The output of the photocells is led to a direct-coupled amplifier which operates the recording device.

In 1944 Hemingway and Taylor[28] constructed a recording oximeter which utilized a new type of high-gain "contact modulated" amplifier developed by Liston and associates,[29] working in the General Motors Laboratories in World War II. Another recording oximeter using this type amplifier was described in 1948 by Hartman, Behrmann, and Chapman,[30] who, with their colleagues,[25, 31, 32] have had extensive clinical and research experience with their "recording oxyhemograph."* Using detecting units of modified design, Elam and associates[21, 22] have utilized the Liston-General Motors amplifiers in their recently developed recording oximeters.

A "condenser modulated" amplifier, employed in the Foxboro "Dynalog Recorder" was employed by the Coleman Instrument Co. in a recording "Anoxia Photometer" manufactured in 1949.† In 1950 we assembled a recording oximeter utilizing, so far as we know for the first time for this purpose, a Brown "Electronik Pyrometer-Potentiometer." This Brown Instrument, also developed during World War II, contains a "chopper" or contact modulated amplifier. A description of the details of construction of this and the other recording oximeters assembled by us follows. The total cost, including labor, of each instrument has ranged from $700 to $2,000. The four units are described in the order in which they were constructed, which happens also to be the order of increasing cost.

UNIT NO. 1. MILLIKAN RECORDING OXIMETER, WITH BROWN ELECTRONIC POTENTIOMETER (FIG. 1)

COMPONENT PARTS.—(1) *Earpiece:* Millikan-Coleman oximeter earpiece,‡ with 10 foot shielded cable and Jones plug No. P-306. (2) *Control Box:* Any

* Manufactured by Photocon Research Products, 1062 North Allen Ave., Pasadena 7, Calif.

† Recording "Anoxia Photometer" manufactured by Coleman Instruments Co,. Maywood, Ill., 1949, and approved in that year by Council on Therapy of A.M.A. No longer in production (as of 1952).

‡ Until recently manufactured and sold with or without associated oximeter control box and galvanometer by Coleman Instruments Co., Maywood, Ill. At present no longer in production though it is hoped that production will be resumed.

small metal cabinet as used for electronic apparatus is suitable. For Unit No. 1 we used a war-surplus metal cabinet 8 by 8 by 4 inches in size to house the relatively few components of the circuit. In Unit No. 2 a commercially available, sloping front cabinet was used, while for Unit No. 3 a special stainless steel cabinet was made to order. (3) *Brown Electronik Potentiometer:** Series 15, Circular or Strip chart instrument; time for full-scale deflection varies with different models from twelve down to two seconds. We were fortunate enough to

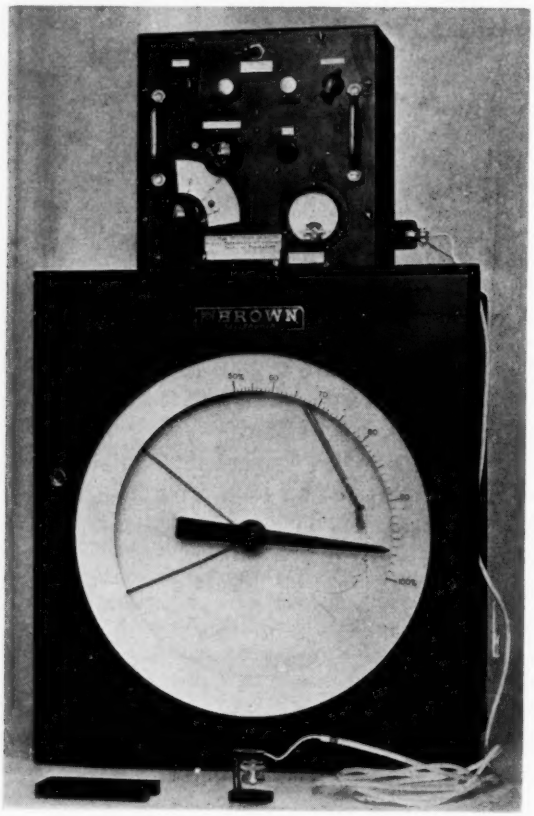

Fig. 1.—Recording oximeter—Unit No. 1. For operation with the Millikan-Coleman earpiece shown, together with the calibration filter, in front of the Brown Electronik Pyrometer. The latter was modified, as described in the text, so as to give full-scale deflection with 500 microvolts.

The unit is mounted on a wheeled table similar to that shown in Fig. 6. A ground wire with heavy spring clip is provided. *Note:* The instructions in the text provide for an oximeter scale running in a direction opposite to that shown for greater convenience in reading the chart.

receive, from another department of the University, a circular chart Brown Electronik Potentiometer Pyrometer which had been used to measure temperatures from 0 to 800 ° C. This was modified as described below. (4) *Wheeled Table:* Any rugged table of wood or welded steel and mounted on casters with large wheels can be constructed (Fig. 6). A drawer for storing the earpiece and other

*Minneapolis-Honeywell Regulator Company, 580 Wayne Ave., Philadelphia 44, Pa. Brown Instrument Division.

components is a useful addition. Suitable wheeled tables originally designed for restaurant or other use are often commercially available (Fig. 8). (5) *Storage Battery, Radio Type:* Exide No. 3LXGH-9, with glass case and flotation balls which indicate degree of charge, has proved satisfactory. An automobile type battery is unsuitable, since it is permanently damaged if thoroughly discharged one or more times. (6) *Battery Charger, Trickle Type:* Mallory No. 6AC4-2 is suitable. The charger must be entirely disconnected from battery and 110-volt A.C. line while the oximeter is in use.

Wiring the Control Box.—Fig. 2 shows the modified Millikan circuit used in Unit No. 1. The original compensated Millikan circuit[10] was developed for use with a galvanometer having a critical damping resistance of 50,000 ohms. Even when modified for high impedance input, the standard Brown instruments will not tolerate input impedances much over 5,000 ohms. For this reason, the Millikan circuit was modified as shown in Fig. 2 so as to present an impedance not greatly exceeding 5,000 ohms to the input of the Brown instrument.

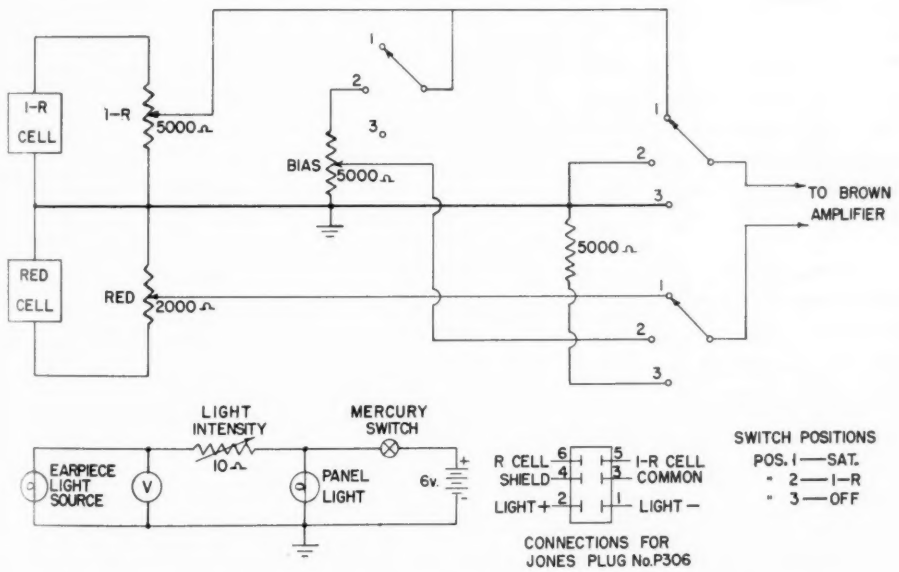

Fig. 2.—Modified Millikan oximeter circuit used in Unit No. 1. It is preferable to double all switch contacts in order to minimize contact resistance. The voltmeter (V) reads from 0 to 6 volts. The earpiece, containing the "I.R." cell, the "Red" cell and "earpiece light source," is shown at the extreme left of the circuit diagram, with its five wires leading directly into the Jones plug, the connections for which are shown.

The component parts, which should be of highest quality, are shown in the circuit diagram. A helical type potentiometer, providing continuous fine adjustment, may or may not be required in the circuit from the "red" photocell. All wiring should be done with especial care, using wire and rosin core solder of high grades in order to minimize spurious potentials and current leaks.

Precautions Against Spark Hazards, if Equipment Is to Be Used With Flammable Anesthetics or Gases.—

Note: The authors of this article hereby assume no liability for spark hazards in equipment built according to these instructions. While certain suggestions for avoidance of spark hazards are given it is assumed that persons directing the construction and assembly of this equipment are familiar with the principles involved in eliminating spark hazards in operating rooms and laboratories and will assume full responsibility for such hazards.

Sparks are not likely to originate in the measuring circuits of the control box or Brown Potentiometer, inasmuch as the voltages involved are of the order of millivolts. Sparks could originate in: (a) the 6-volt circuit leading from the storage battery to the lamp in the earpiece, (b) the 110-volt, A.C. circuit leading to the Brown instrument or other amplifying and recording devices. The precautions taken against sparks were as follows: (a) *6 volt lamp circuit:* Heavy, rubber-covered lamp cord was soldered (not clamped) directly to the terminals of the storage battery and was then led through a rubber grommet in the back of the control box to the appropriate terminals inside. For turning the earpiece lamp on and off, a glass-enclosed mercury switch was used. A metal clamp was provided on the back of the control box to hold the Jones plug securely in place. The earpiece cord should be checked periodically for possible breaks in the 6-volt circuit. (b) *110-volt, A.C. circuits to Brown instruments:* The Brown amplifier was connected permanently to the 110-volt power cord. A mercury switch was inserted in the circuit to the motor which drives the chart. The 110-volt power leading from the instrument was plugged into and securely taped to a safety-type extension cord approved for operating room use.

Modification of Brown Class 15 Electronik Instruments for Use With Recording Oximeters.—Since many "Class 15" Brown instruments are in use in various laboratories in this country, not only for measuring temperatures but for various other purposes, we are describing the several steps necessary to convert them into amplifier-recorders for oximetry. They may readily be reconverted to their original functions if desired.

Because they are not available in simplified form from the Brown Company, the following instructions, derived partly from the 350 page Brown Service Manual No. 15019M and Brown Bulletin No. B15-10, partly from local service representatives of the Company, and partly from personal experimentation, are included here. Though our own experience has been limited to the pyrometer-potentiometer type of Brown instruments, it should be possible also to adapt the resistance thermometer instruments for recording oximetry by means of information furnished by the Brown Company.

(1) *Increasing Sensitivity of Standard Amplifier:* The input transformer and first stage of the Brown amplifier are shown in Fig. 3, *A*. The sensitivity of the amplifier is increased by removing resistor R-13 and changing condenser C-1 to 0.003 MFD.* The amplifier can be removed from the cabinet without disconnecting the flexible cables which lead into the amplifier.

*For those desiring utmost sensitivity, the value of this condenser can be determined somewhat more precisely by tuning the circuit to 60 cycles according to the principles of resonant circuit theory.

(2) *Increasing the Input Impedance:* A damping circuit is employed in the input of the Brown instrument as shown in Fig. 3, *B*. When it is desired to modify the instrument for an input impedance of 5,000 ohms, the circuit shown in Fig. 3, *C* should be used. In the circular chart instruments, C_D and R_D, damping condenser and resistor, respectively, are located under a removable plate near the standardizing switch at the upper left portion of the instrument.

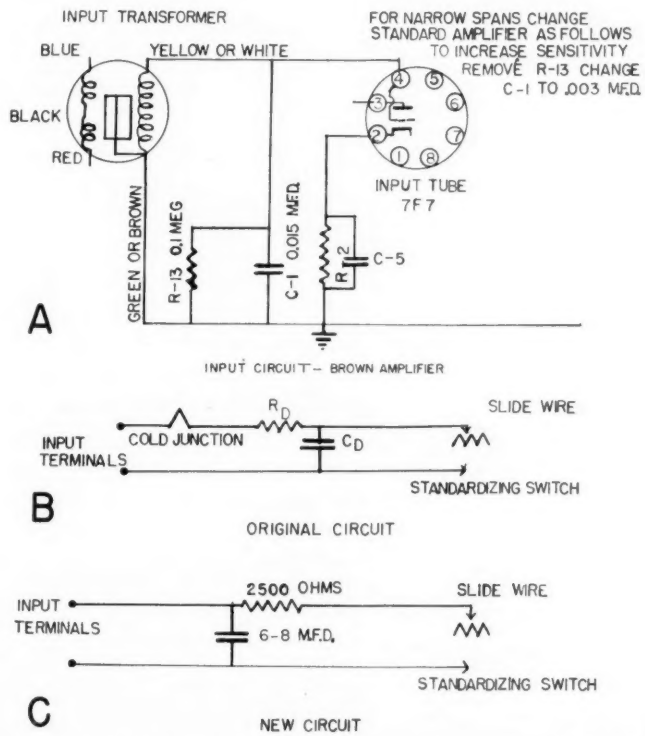

Fig. 3.—*A*, Simple modifications necessary for increasing the sensitivity of the standard Brown amplifier. Resistor No. R-13 is removed, and condenser No. C-1 is changed to 0.003 MFD. This portion of the amplifier may be identified by locating the leads from the input transformer as they pass to the input tube, No. 7F7. *B*, Original input to measuring circuit for damping and compensating Brown instrument for use with thermocouples. The modifications described in the text include removing the cold junction and changing the value and position of Resistor "R_D" and the value of condenser "C_D," as shown in Fig. 3,*C*. *C*, Modified input circuit which permits usnig Brown instrument with an input source measuring 5,000 ohms.

In the strip chart instruments these parts are located on the rear of the input terminal block. A good quality oil-filled (not electrolytic) condenser is to be substituted for C_D. If possible, this condenser should be selected by testing with a sensitive galvanometer so as to be free of potentials in excess of 2 to 3 microvolts. Because of its relatively large size, this condenser will probably have to be placed in a new location within the cabinet. A radio type, or wire-wound resistor can be used for R_D.

(3) *Conversion of Instrument From Pyrometer (Temperature Measuring) to Millivolt Operation:* The reference "cold" junction resistor, attached by four screws to the input terminal block and shielded by a metal cover, should

be removed. In its place there should be inserted an uncompensated resistor spool, resistor part No. 78130-6. A manganin precision resistor of 5.0 ohms can be substituted for this part if desired. This resistor is to be connected between the pair of terminals originally connected to the right end of the reference junction spool. A wire jumper should be placed between the pair of terminals originally connected to the left end of the spool.

For multiple point instruments wired with thermocouple wire, it is necessary to bypass the input terminals and selector switch. This is done by removing the two wires leading from the selector switch from their terminals on the rear of the input terminal block, and bringing the input leads to this point.

(4) *Narrowing the Span of the Input Voltage:* The voltage required to produce full-scale deflection of the instrument is determined by a set of five measuring circuit resistors. These are marked with their resistance, numbered, and mounted in the correspondingly numbered position on a panel. In circular chart instruments this panel is mounted under a removable cover in the left front part of the instrument, and in strip chart recorders under a removable cover at the top of the instrument. The total span of the instrument is to be reduced to 0.5 millivolt, provided this can be accomplished without sacrifice of stability of operation. Formulas for calcuating the exact value of the measuring circuit resistors are given on page 24, section 1550 of the Brown Service Manuel, No. 15019M. Appropriate values for these resistors have been calculated so as to provide full-scale operation with an input of –0.5 to 0 millivolt. By this arrangement, both the circular and strip charts of the Brown instruments can be read (like a conventional graph) from left to right, high values up. This means that the pens of the circular and strip chart instruments are at full right position when the input voltage is zero. The resistors, their functions and values for –0.5 to 0 millivolt full-scale operation are shown in Table I.

TABLE I.

Resistor Designation	*Function*	*Resistance in ohms*
R_o Resistor No. 1	Determines position of pointer at lower end of span	2.3738
R_t Resistor No. 2	Determines width of span	0.13378
R_a Resistor No. 3	Determines position of pointer at upper end of span	254.74
R_c Resistor No. 4	For standardizing—same in all instruments	509.5
R_n Resistor No. 5	Standardizing shunt	3.9
R_f Resistor No. 6	Present only in models with "Downscale Burnout" feature	10 megohms

Resistor No. 4 need not be changed. Resistor No. 5 is a radio type resistor whose value is not critical. Resistor No. 6, if present, should be removed. The extremely precise resistors used in positions Nos. 1, 2 and 3 can be made to order by the Brown Company (delivery one to three months) and can be supplied in new instruments upon request. In older instruments, however, there are certain advantages to be gained by utilizing resistors whose values merely approximate those given, adjusting these values as described below. Thus, the operation of the recording oximeter does not require that the instru-

ment be calibrated precisely in millivolts. Moreover, since they were not designed to operate with such narrow spans, some of the older Brown instruments may fail to show stable operation when the span is narrowed to only 0.5 millivolt. Since the output of the modified Millikan circuit may actually exceed one millivolt, for the Millikan instrument the span may not need to be narrowed below that figure. However, for Wood oximeter operation, a span of 0.5 millivolt or less is necessary in order to obtain full-scale deflections.

For these reasons, we suggest that IRC manganin wire resistors be substituted for resistors Nos. 1, 2, and 3. If the resistance of each is made somewhat greater than specified, a precise adjustment can be obtained by means of rheostats shunted across each resistor. Once the necessary adjustments are completed, a second manganin resistor of suitable value can be substituted for the rheostat. Additional adjustment of the span voltage is provided by the span adjustment slidewire located near the resistor panel.

If the instrument, when properly grounded, proves unstable after making these modifications, improvement is often obtained by reversing the 110-volt A.C. plug in the wall socket. It may also, in some instances, be worth while to purchase an input transformer, specially selected by the Brown Company for operation with high input impedances.

The output of the control box should be connected to the input of the Brown instrument by a shielded cable containing two leads. Neither of these leads is grounded when the oximeter is set to "saturation," though the common photocell lead is grounded in the control box. This arrangement provides for greater stability of operation than if one side of the input were grounded.

Operation of Millikan Recording Oximeter.*—(1) *Construction of Oximeter Scale:* The scales of the Millikan and single-scale Wood oximeters are logarithmic. They may be constructed as follows: Swing an arc with its center at number 4 on the abscissa of large, single cycle logarithmic paper. The radius of the arc should equal the length of the desired oximeter scale. Allow the arc to cut the vertical line above No. 9. Draw a line between the center of the arc and its intersection on the vertical. The major scale divisions are given by the intersections of this oblique line upon the verticals above Nos. 4 through 9. One hundred per cent is at No. 4, 90 per cent is at the intersection above 5, and so on down to 50 per cent which is above 9. For the Wood single-scale oximeter a scale from 100 to 40 per cent may be produced by swinging the arc between Nos. 4 and 10. It should be emphasized that this theoretical logarithmic relation between per cent saturation and instrumental readings may diverge considerably from an empirical calibration based on Van Slyke analyses.

In keeping with the modifications described for the Brown instrument, 100 per cent saturation is to be at the full counterclockwise position of the pointer, left and down position of the pen on circular chart instruments, and at

*Instructions modified from description of single-channel oximeter, C.M.R. Model 13, by Glenn Millikan, issued by the Johnson Foundation, Philadelphia, Pa., 1944, and from the article by Earl H. Wood on Oximetry, Medical Physics, Vol. II, edited by Otto Glasser, Chicago, Medical Year Book Publishers, 1950.

full left position in strip chart instruments. Actually, it is best to leave a slight space beyond 100 per cent for the occasional instances when an oximeter reads one to three per cent over 100 per cent due to the inherent limitations of its accuracy.

(2) *Setting the Millikan "Bias" Potentiometer:* Place the "ear thickness" filter Coleman No. 17-20 in the earpiece and turn on the earpiece lamp, adjusting voltage to 5.3. Set selector switch to "I.R." When the "I.R." potentiometer is turned clockwise, the pointer of the Brown instrument should move toward 100 per cent. If it moves in the other direction the polarity of the leads from the control box should be reversed to obtain correct polarity.

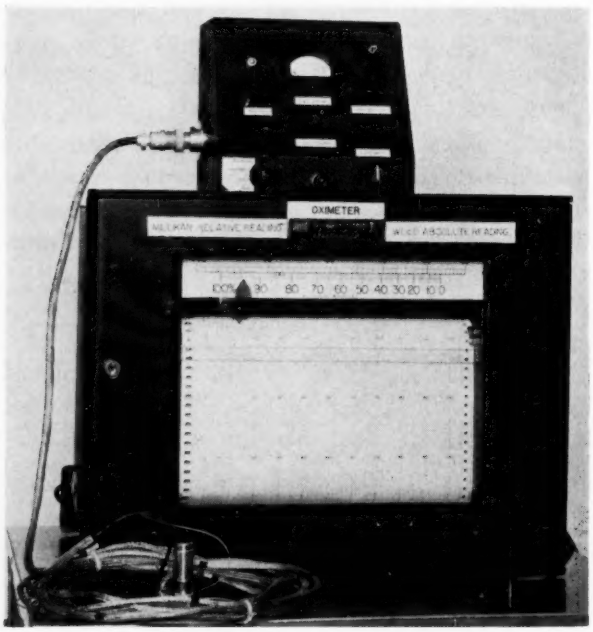

Fig. 4.—Recording oximeter No. 2. For Millikan (relative reading) or Wood (absolute reading) operation. A Wood-Waters-Conley oximeter earpiece, with Cannon plug connecting it to the control box, is shown. A flange was specially constructed for mounting the male portion of the Cannon plug to the chassis. The following precautions have been taken to eliminate spark hazards: the halves of the Cannon plug are locked together by means of the threaded ring provided with the plug. Sealed mercury switches are used to operate the earpiece light source and the chart-drive of the Brown instrument, whose amplifier is permanently connected to the 110-volt A.C. line cord. The switch for selecting either Millikan or Wood operation does not show in the photograph because it is located on the right side of the control box, as is the Jones plug for the Millikan-Coleman earpiece. The latter is locked to the chassis by a specially constructed clamp.

The "bias" potentiometer is to be adjusted so that with the selector switch on "I.R." the voltage delivered to the instrument from the "I.R." cell is one-fifth of that delivered when the selector switch is on "SAT." This is accomplished as follows: (a) Reverse the polarity of the leads from the control box. (b) Set selector switch on "SAT." (c) Turn the "red" potentiometer full counterclockwise (zero output). (d) With the filter in place and lamp on, turn the "I.R." potentiometer clockwise until the pointer moves full scale. Leave the setting of the "I.R." potentiometer unchanged. (e) Reverse the polarity of the leads once more (correct polarity resumed). (f) Set selector switch to "I.R."

(g) With a screwdriver, adjust the "bias" potentiometer until the pointer moves one-fifth of the linear length of the scale. The "bias" potentiometer should be permanently locked in this position.

(3) *Use of Millikan Oximeter on Subject:* (a) With filter in earpiece, lamp on, voltage 5.3, set selector switch to "I.R." (b) Open "I.R." potentiometer until pointer moves to 100 per cent. (c) Turn off lamp and apply earpiece to pinna of ear. Prior to application, rub ear in order to render hyperemic. (d) Turn on lamp, allow ear to warm for 5 to 10 minutes. (e) Set selector switch to "SAT." (f) Adjust "red" potentiometer until instrument reads 97 per cent (subject breathing air) or 100 per cent (subject breathing oxygen).

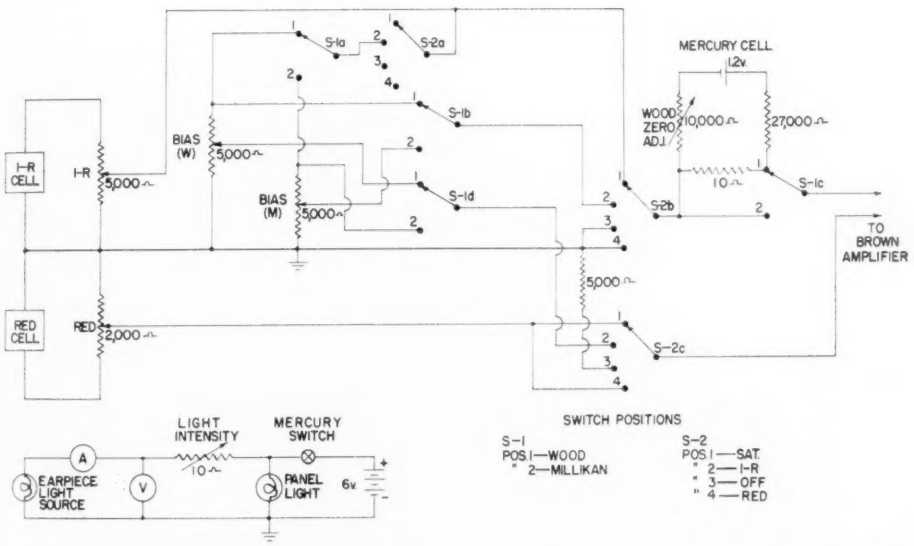

Fig. 5.—Modified Millikan and Wood oximeter circuits as used in control box of recording oximeter No. 2. When changing from Millikan to Wood operation, the pointer of the instrument must be moved from the end of the scale corresponding to low saturation, to near the high end. This is effected by applying a potential from a mercury cell hearing aid "A" battery (Mallory No. RM-3), by means of the resistance network shown (upper right). The connections for the Jones plug (for Millikan earpiece) are given in Fig. 2, and for the Cannon plug (Wood earpiece) in Fig. 7.

RECORDING OXIMETER NO. 2. (FIG. 4) MILLIKAN OR WOOD OPERATION, BROWN ELECTRONIK POTENTIOMETER

COMPONENT PARTS.—(1) *Earpieces:* (a) Millikan-Coleman oximeter earpiece (see unit No. 1) and/or (b) Wood-Waters-Conley oximeter earpiece* provided with 10 foot shielded cable and cannon No. WK-6 plug. (2) *Control Box:* The circuit (Fig. 5) provides for operation as a Millikan or a Wood single-scale oximeter. For Wood operation, the mechanical zero must be shifted to the end of the scale corresponding to high saturation. This is done by introducing a

*The Waters-Conley Co. Rochester, Minn., have agreed to sell the Wood type oximeter earpiece, manufactured by them, with or without associated control boxes and galvanometers as desired.

†A special high gain "40×" amplifier can be added to new Brown Instruments during manufacture, or purchased for addition to older models. This provides for 100 microvolts full-scale operation. Though we have had no experience with it, the resulting highly sensitive Brown instrument should be satisfactory for Wood oximeter operation. We have been told by representatives of the company that careful shielding against 60 cycle pickup is necessary.

potential, which can be varied, from a small mercury cell battery, as indicated in the circuit diagram. Other components are similar to those used in unit No. 1. (3) *Amplifier-recorder†:* A strip-chart Brown Potentiometer was purchased especially for this application, with –0.5 to 0 millivolt span, 5,000 ohms input impedance. The necessary modifications, as outlined above, were introduced during construction at the factory.

Calibration and Operation of Wood Single-Scale Oximeter.—A set of filters for calibration is included with each Wood type oximeter earpiece sold by the Waters-Conley Company, together with instructions for calibrating and operating the instrument. An abbreviated form of these instructions, modified so that the Wood single-scale oximeters may be used with recording equipment, is presented here.

(1) *Initial Settings:* The basic procedure for making the initial settings of the single-scale Wood oximeter for each subject is as follows.[33] With the earpiece in place and lamp on, the selector switch is turned to "I.R." The output of the "I.R." photocell is adjusted to a standard "I.R. setting" voltage, which is the same for all subjects. It makes little difference what the saturation of the blood happens to be during this procedure, because the output of the "I.R." photocell is virtually unaffected by changes in oxygen saturation.

The selector switch is now set to the "saturation" position and the ear is rendered bloodless by inflating the capsule to a pressure of 200 mm. Hg. The output of the "red" photocell is adjusted so as to bring the indicator of the instrument back to the mechanical zero point. In this situation the two photocells have cancelled one another, resulting in zero "difference" voltage. This completes the necessary initial settings. When the pressure in the capsule is released, the instrument provides absolute readings of oxygen saturation on a previously calibrated scale.

(2) *Construction and Calibration of Scale for Wood Single-Scale Oximeter:* Although the Wood instrument performs satisfactorily on colored subjects, the resulting voltage output is small. For this reason, it has not been found possible to obtain full-scale deflections for all subjects on the Brown instruments used. Once the gain of a given Brown instrument has been increased to the limit of its stability, it is necessary to determine the maximum possible length and range of the Wood single oximeter scale, as follows: (a) using zero adjusting rheostat in control box (see circuit diagram, Fig. 5), set "mechanical zero" at a point roughly 80 per cent of the linear distance from the low to the high end of the scale. (b) Apply earpiece to ear of a subject whose skin is estimated to be as heavily pigmented as that of most individuals on whom the instrument may later be used. Turn on lamp. (c) Set selector to the "I.R." position and open "I.R." potentiometer to approximately 90 per cent of full output. (d) With screwdriver, adjust "bias" potentiometer until pointer reaches an "I.R. setting" mark located arbitrarily at a point just short of the low end of the scale. Lock the "bias" potentiometer.

Procedures (a) through (d) above serve to establish the "I.R. setting" voltage. Thereafter, the output of the "infrared" cell will be adjusted initially

to this value on all subjects. This value determines the length of the oximeter scale. This is because, with a large "I.R. setting" voltage, a large fraction of the output of the red photocell is required to cancel out the voltage of the "infrared" cell during the initial setting procedure. Changes in oxygen saturation will therefore produce relatively large changes in the voltage contributed to the circuit by the "red" cell, leading to an expanded oximeter scale. If subjects with heavily pigmented skins are anticipated, the "I.R. setting" voltage will be small, and a shorter oximeter scale must be used, unless a very high gain amplifier is available (see Units Nos. 3 and 4).

With the "I.R. setting" voltage established and "bias" adjustment locked, the oximeter scale may now be constructed and calibrated with the help of the

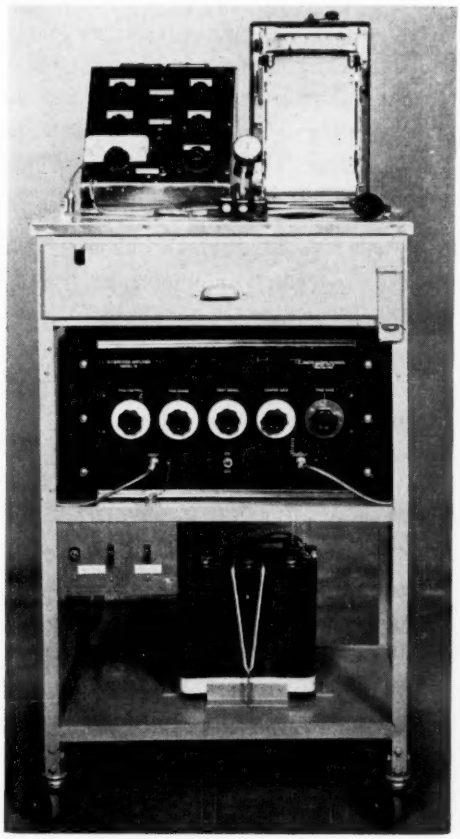

Fig. 6.—Recording oximeter No. 3. For Wood-Waters-Conley earpieces or cuvette, single- or double-scale operation. Next the stainless steel control box on the wheeled table is the Esterline-Angus recorder with oximeter scales running from 100 to 40 per cent and from 100 to 0 per cent. The Liston-Folb contact modulated amplifier is on the middle shelf, and the glass storage battery, with flotation balls to indicate charge, is on the lower shelf. The mercury switches for turning on the amplifier and the chart-drive of the recorder are located to the left of the battery, and control the 110-volt power to a double outlet box, to which are clamped the 110-volt plugs from amplifier and recorder. Final precautions against spark hazards include permanent shortcircuiting of original power switches on amplifier and recorder.

On the table in front of the control box is a Wood-Waters-Conley infant type earpiece, together with its three calibration filters and the rubber bulb and pressure gauge for inflating the capsule of the earpiece.

special filters provided with the earpiece. The initial settings are repeated with the appropriate filters in place, as outlined in the Waters-Conley Instruction Manual. With the selector set to "SAT.," the position of the pointer on the original millivolt scale of the Brown instrument is determined for each of the three filters. These positions, with corresponding values of oxygen saturation as given for each filter in the Waters-Conley manual, are transferred to a strip of cardboard. By placing the cardboard obliquely on a sheet of logarithmic graph paper, and manipulating it to a certain position, the scale divisions between 100 and 50 per cent may be marked on the cardboard (see construction of oximeter scale in description of Unit No. 1 above). Alternatively, the Wood scale, range 100 to 0 per cent, which is printed in the Waters-Conley folder describing their "Simplified Absolute Reading Ear Oximeter" may be enlarged by suitable means. It is recommended that two oximeter scales be employed, one ranging from 100 to 50 per cent for lightly pigmented subjects, the other ranging from 100 to 0 per cent for heavily pigmented subjects. For the latter a separate "I.R. setting" mark, located to the left of the first "I.R. setting" will be required. The scale ranging from 100 to 50 per cent is also to be used when operating the instrument as a Millikan oximeter.

RECORDING OXIMETER NO. 3. (FIG. 6) FOR OPERATION WITH WOOD EARPIECE OR CUVETTE. LISTON AMPLIFIER, ESTERLINE-ANGUS RECORDER

COMPONENT PARTS.—(1) *Earpieces*:* (a) Wood type adult earpiece, with shielded 10 foot cable and Cannon plug, No. WK-6. (b) Wood type infant earpiece (optional), with shielded cable and plug. (c) Wood type cuvette for whole blood (optional), with shielded cable and plug. (2) *Control box:* Specially constructed of stainless steel, lacquered with Krylon plastic. The circuit diagram (Fig. 7) permits use of either of the earpieces or the cuvette as desired, for Wood single or double scale operation.

A six position selector switch, employing 10 wafers, permits doubling all switch contacts for greater reliability. One side of the amplifier is internally grounded. This internal ground of the amplifier is not connected to the same point as are the cable shields and cases of the amplifier and control box. For this reason, the oximeter circuit is not grounded within the control box, though one side of the lamp circuit is so grounded. A concentric input plug providing three circuits is included with each amplifier, together with shielded input cable and low-noise solder. (3) *Liston Type Amplifier†:* We used a Model 10 Liston-Folb amplifier, contact modulated at 8 cycles per second and with 10,000 ohms input impedance. Frequency response of this amplifier is flat to approximately one cycle per second. A frequency response essentially flat to 10 cycles per second is obtained in the Model 10A Liston amplifiers contact modulated at 80 cycles per second. There is somewhat more likelihood of having difficulty with

*Waters-Conley Co., Rochester, Minn.

†Liston-Folb, now Liston-Becker Instruments Co., Inc., P.O. Box 1110, Stamford, Conn. Model 14 Liston-Becker amplifier replaces model 10 Liston-Folb.

60-cycle pickup in the latter units. (4) *Esterline-Angus Recorder*:* Model A.W., 0-5 milliamperes full scale; number of seconds required to swing pen one way = 0.5.

These units were mounted with battery and battery charger on a wheeled table as shown in Fig. 6. The 110-volt power switches in amplifier and chart-drive of recorder were permanently short-circuited to avoid spark hazards. Two enclosed mercury switches, located in a small case on the lower front section of the table, were provided for turning amplifier and chart-drive on and off.

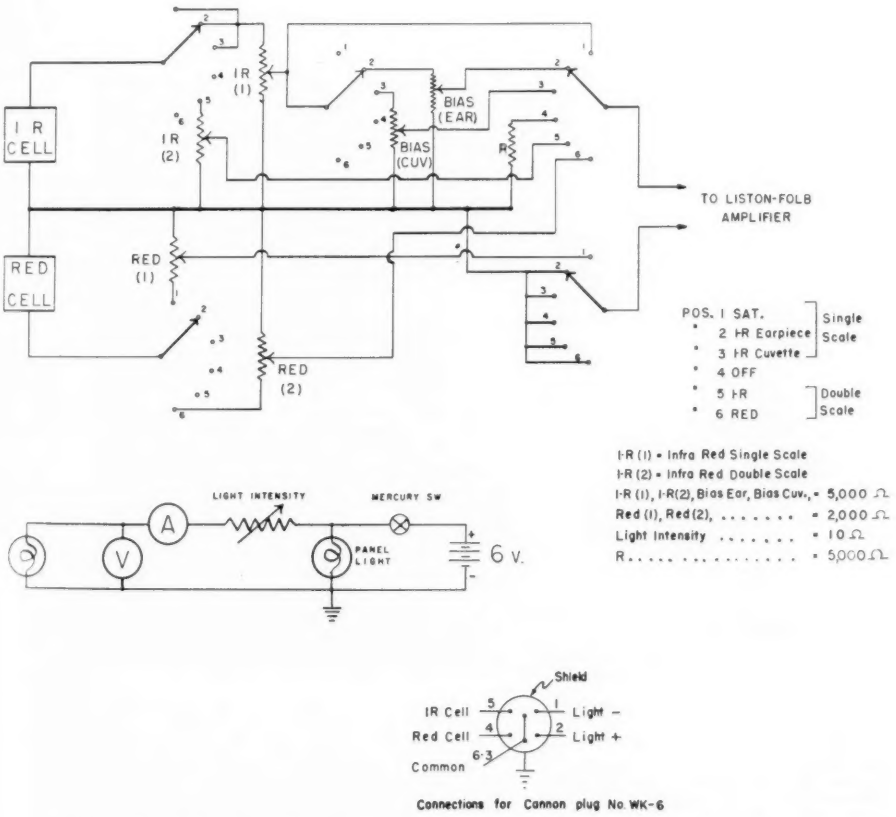

Fig. 7.—Modified Wood oximeter circuit for Unit No. 3. The oximeter circuit is grounded within the Liston-Folb amplifier and not in the control box.

Operation of Recording Oximeter No. 3.—The gain of the amplifier has usually been set at 16.5 (maximum 21) leaving extra amplification for expanding the scale as desired. Wood single oximeter scales running the length of the recorder scale were constructed and attached to the recorder. One scale ranges from 100 to 40 per cent and is used on human subjects only. The other scale, from 100 to 0 per cent has been used on experimental animals, and occasionally on chronically cyanotic human subjects. Each earpiece and the

*The Esterline-Angus Co., Indianapolis, Ind.; also local representatives.

cuvette for whole blood were calibrated so as to give readings corresponding to those on the oximeter scales, according to the principles of calibration outlined under description of Unit No. 2. For the earpieces, the special calibration filters were used. Van Slyke analyses were used in calibrating the cuvette, which was connected in an experimental animal to an artery and vein so as to allow blood to flow continuously through it. The mechanical zero of the Esterline-Angus recorder was set manually to the point on the oximeter scale specified in the Waters-Conley instruction manual. The bias setting was determined by trial-and-error adjustment until the appropriate scale readings were obtained for each calibration filter. Since only one "bias" potentiometer is provided for

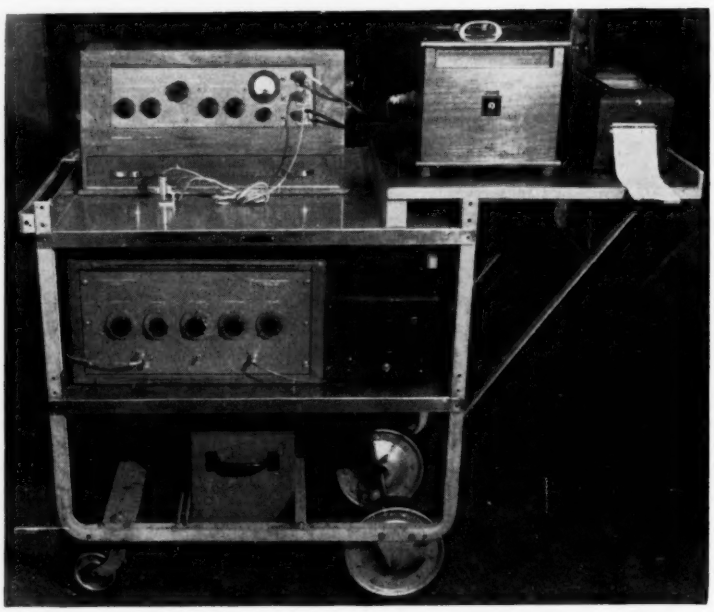

Fig. 8.—Recording oximeter No. 4. The Wood-Waters-Conley earpiece, control box and galvanometer are on top of the table. A selector switch was added at the right end of the control box for switching to recording operation using the Brush recorder (located to the right of the galvanometer). The Liston-Folb amplifier, to the right of which is the special D.C. amplifier (see Fig. 9), is located on the shelf, with the battery below. The wheeled table was obtained from a restaurant supply firm.

all the Wood type earpieces, individual "I.R. setting" marks for each earpiece were placed on the oximeter scale. One of these marks can, for convenience, be placed at the end of the scale, as can the "I.R. setting" mark for the cuvette which has its own "bias" potentiometer. The use of the gain control on the amplifier has been found to facilitate the process of calibration.

For double-scale operation, the selector switch is set alternately to the double-scale positions labelled respectively "red" and "I.R.," and readings are made according to the procedure described by Wood and Geraci[18] which is also given in the Waters-Conley Manual for the Oximeter.

RECORDING OXIMETER NO. 4. (FIG. 8) FOR WOOD OPERATION WITH BRUSH RECORDER

COMPONENT PARTS.—(1) Complete Waters-Conley galvanometer-type Wood oximeter manufactured in 1950 for single or double scale operation with earpiece or cuvette. Shielding was applied to the cable of the earpiece. (2) Liston amplifier, preferably 80 cycle type. (3) Supplementary D.C. amplifier (see below). (4) Brush recorder.* (5) Associated equipment.

OPERATION.—A switch was added to the Waters-Conley control box so that the output of the single-scale oximeter circuit could be led to the Liston amplifier. A supplementary direct-coupled amplifier was required to increase the output of the Liston amplifier sufficiently to drive the Brush recorder. This amplifier (Fig. 9) was specially designed (S.H.B.) and built so as to have its output

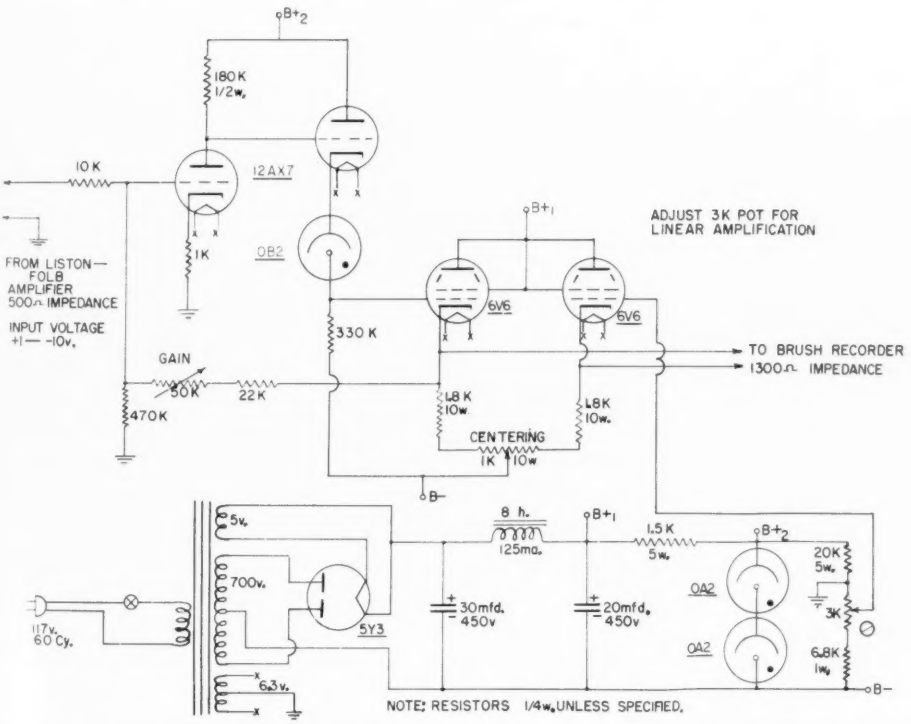

Fig. 9.—Circuit of special D.C. amplifier for operating Brush recorder from Liston-Folb amplifier.

voltages operating on either side of ground, rather than well above ground as in conventional push-pull amplifiers. This feature was considered desirable because the pen of the Brush recorder was connected to one end of the coil which drives the pen. By using a Liston amplifier modulated at 80 cycles per second a frequency response up to 10 cycles per second could be obtained, though greater precaution would have to be taken in shielding control box against stray 60 cycle pick-up.

*Brush Development Co., Cleveland, Ohio.

RESULTS

(a) *Accuracy.*—We have made no systematic studies correlating oximeter readings with Van Slyke analyses in human subjects. We have assumed the Wood calibration filters to be sufficiently accurate for our requirements. Readings of the single scale Wood oximeter have ranged from 97.5 to 103 per cent with a limited number of normal subjects breathing pure oxygen. The oximeter cuvette was calibrated by means of Van Slyke analyses.

Possible Increases in Accuracy Due to Modifications Introduced into the Circuits Used: Though we do not have experimental proof, we believe that the modifications we have made on the Millikan and Wood oximeter circuits so as to adapt them for use with recording instruments has by chance tended to increase the accuracy of these instruments. Thus, Wood[19] (pages 14 and 15) states that "if the single scale circuit is to operate as designed, it is necessary that the output of the photocells be independent of the variations in their external load resistance encountered under operating conditions. Actually this has been found not to be the case." It so happens that variations in the external load resistance of the photocells have been greatly minimized in two of our recording oximeters and entirely eliminated in the other two. This is because each photocell is connected to the end of the resistance wire of the output potentiometer rather than to the sliding contact, and because the recording instruments have high input impedances (10,000 ohms for Liston amplifier, impedance infinite for Brown Potentiometer at balance). This arrangement also minimizes a second possible source of error, namely, an influence of the output of the "red" cell upon the output of the infrared cell.[24]

Possible Sources of Error: Units Nos. 1 and 2, using the Brown instruments as modified for recording oximetry may show "dead space," up to 1 to 2 per cent of full scale. "Dead space" refers to the fact that the indicator comes to rest at a slightly different point when moving from the left than when moving from the right. No measurable "dead space" is present in Units Nos. 3 and 4.

(b) *Uses of the Recording Oximeters.*—As stated in the introduction, these recording oximeters have had extensive use during the past two years. Unit No. 1 is used to correlate, during classes in mammalian physiology, changes in oxygen saturation in students undergoing anoxia or asphyxia with changes in respiratory rate and volume, and for other class experiments on human subjects or animals. Units Nos. 2 and 3 are used frequently in the University of Chicago Clinics during prolonged operative procedures, especially those involving opening of the chest, upright posture of the patient, and operations on patients who are poor surgical risks.[5] Fig. 10 is an unretouched reproduction of a Millikan oximeter record made during a valvulotomy for pulmonic stenosis. The rapid rise of the oxygen saturation to a satisfactorily high level gave the surgeon an indication that further, possibly hazardous, cutting of the pulmonic valve was not necessary. Table I summarizes the results of the use of the oximeters during operative procedures.

At the present time Unit No. 4 is being used by members of the Department of Pediatrics for measuring cardiac output by means of the Nicholson, Burchell,

and Wood dye method.[3] Unit No. 4 will shortly also be used on studies of newborn infants.

As mentioned previously, we have applied the recording oximeters to studies of pulmonary function. We have devised a test, the "In-Vivo Transpulmonary Oxygen Dissociation Curve,"[6] which involves using recording oximeters to measure the oxygen saturations corresponding to various oxygen

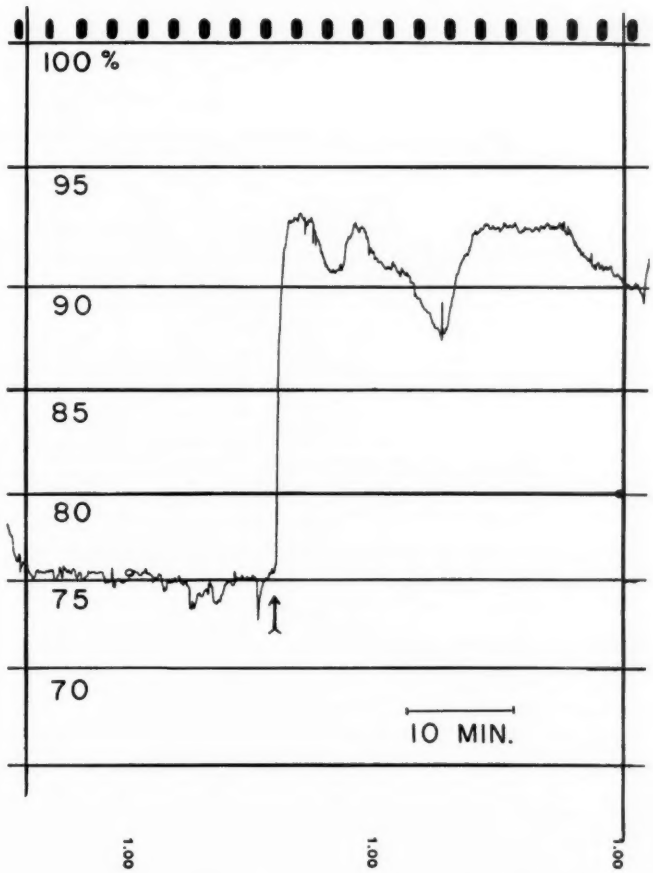

Fig. 10.—Unretouched record made by Recording oximeter No. 2 during a valvulotomy operation for pulmonic stenosis. The pulmonic valve was cut at the time marked by an arrow. The rapid rise in oxygen saturation to a satisfactorily high level indicated to the surgeon that further, possibly hazardous, cutting of the valve was unnecessary.

tensions of alveolar air, as sampled by Rahn alveolar sampler and analyzed by Pauling oxygen meter. We believe that this test, which can be carried out on a patient in about one hour, may be useful in evaluating the degree of shunting of blood through the lung, whether through aberrant blood vessels or through poorly aerated alveoli. It may also be helpful in evaluating loss of effective area for diffusion in the lung.

We are at present engaged in studies of pulmonary function in animals. Fig. 11 shows the effect of injecting a rather large dose of Nembutal into a dog whose saturation was being measured by Unit No. 3 connected to a Wood type cuvette through which blood flowed continuously, from an artery to a vein. Prior to the injection, shifts in oxygen saturation of less than 1 per cent were seen with each breath, but with the slowing of respiration which followed the Nembutal, the saturation fluctuated 15 to 20 per cent with each breath.

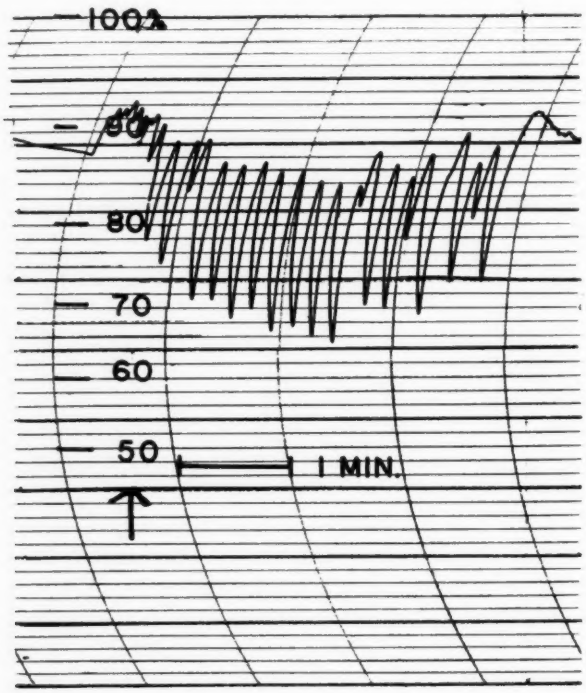

Fig. 11.—Shifts in oxygen saturation correlated with respiration in a dog. The Wood oximeter cuvette was used, with blood flowing continuously through it from carotid artery to jugular vein. At the time marked by an arrow a relatively large dose of Nembutal was injected, causing a slowing of respiration from 15 to 18 breaths per minute down to approximately 5 breaths. Prior to the injection the shifts in saturation with each breath were of the order of one per cent, but rose to 15 to 20 per cent with the slowing in respiration.

SUMMARY

During the past two years, with the assistance of student technicians, four recording oximeters have been assembled. Two are of the Millikan "relative reading" type, and two are of the Wood "absolute reading" type. These instruments have been used on over 250 patients during surgery, in studies of pulmonary reserve in patients and animals, and in teaching respiratory physiology. Recording oximeters possess the following advantages over galvanometer type oximeters, namely, ruggedness, ready visibility, continuous automatic recording in ink, and (in certain models) increased speed of response.

TABLE I. USE OF RECORDING OXIMETERS DURING VARIOUS TYPES OF SURGICAL OPERATIONS UNIVERSITY OF CHICAGO CLINICS, 1950-1952

Thoracotomies	
Pulmonary Surgery (including exploratory thoracotomy, lobectomy, pneumonectomy)	113
Mediastinal surgery (including esophagus, tumors, cysts)	17
Cardiac surgery (including congenital heart defects)	25
Extrapleural Operations	
Thoracoplasty, etc.	28
Laparotomies	30
Cervical operations (including thyroid, radical neck dissection)	5
Laminectomies	14
Endoscopies	
(Bronchoscopy, esophagoscopy)	9
Total	241

This communication presents specific and detailed instructions for converting Millikan and Wood oximeters into direct-writing recording instruments at minimal cost.

We wish to thank Dr. Earl H. Wood for his he'pful advice and suggestions.

REFERENCES

1. Adams, W. E., Thornton, T. F., Jr., and Eichelberger, L.: Cavernous Hemangioma of the Lung (Arteriovenous Fistula), Arch. Surg. **49:** 51-58, 1944.
2. Charbon, B. C., Adams, W. E., and Carlson, R. F.: Surgical Treatment of Multiple Arteriovenous Fistulas in the Right Lung in a Patient Having Undergone a Left Pneumonectomy Seven Years Earlier for the Same Disease, J. Thoracic Surg. **23:** 188-196, 1952.
3. Nicholson, J. W., III, Burchell, H. B., and Wood, E. H.: A Method for the Continuous Recording of Evans Blue Dye Curves in Arterial Blood and Its Application to the Diagnosis of Cardiovascular Abnormalities, J. LAB. & CLIN. MED. **37:** 353, 1951.
4. Harper, P. V.: Use of the Oximeter to Determine Saturation of Blood With Carbon Monoxide, Unpublished data.
5. Livingstone, H. M., and Adams, W. E.: The Value of Oximetry During Anesthesia in Patients With Limited Pulmonary Reserve, To be published in Anesth. & Analg. July-August issue, 1952.
6. Perkins, J. F., Jr., Adams, W. E., Christoff, N., Flores, A., and Webber, W. W.: Tests of Pulmonary Function Using Recording Oximeters, Fed. Proc. **11:** 121, 1952.
7. Nicolai, L.: Über Sichtbarmachung, Verlauf und Chemische Kinetik der Oxyhämoglobinreduktion im Lebenden Gewebe, Besonders in der Menschlichen Haut, Arch. f. d. ges. Physiol. **229:** 372-384, 1932.
8. Millikan, G. A.: A Simple Photoelectric Colorimeter, J. Physiol. **79:** 152-157, 1933.
9. Millikan, G. A.: Experiments on Muscle Haemoglobin in Vivo; the Instantaneous Measurement of Muscle Metabolism, Proceedings of the Royal Society of London, series B. **123:** 218-241, 1937.
10. Millikan, G. A.: The Oximeter, an Instrument for Measuring Continuously the Oxygen Saturation of Arterial Blood in Man, Rev. Scient. Inst. **13:** 434-444, 1942.
11. Kramer, K.: Fortlaufende Registrierung der Sauerstoffsättigung im Blute an Uneröffneten Blutgefässen, Klin. Wchnschr. **13:** 379-380, 1934.
12. Matthes, K.: Über den Einfluss der Atmung die Sauerstoffsättigung des Arterien blutes, Arch. f. Exper. Path. u. Pharmakol. **176:** 683-696, 1934.
13. Matthes, K., and Gross, F.: Untersuchungen über die Absorption von Rotem und Ultrarotem Licht durch Kohlenoxydgesättiges, Sauerstöffgesattigtes und Reduziertes Blut, Arch. F. Exper. Path. u. Pharmakol. **191:** 369-380, 1939.
14. Brinkman, R., and Wildschut, A. J. H.: A Clinical Method for Rapid and Accurate Determination of Oxygen Saturation in Small Amounts of Blood, Acta Med. Scandinav. **94:** 459-466, 1938.
15. Squire, J. R.: An Instrument for Measuring the Quantity of Blood and Its Degree of Oxygenation in the Web of the Hand, Clin. Sc. **4:** 331-339, 1940.

16. Hartman, F. W., and McClure, R. D.: Further Anesthesia Studies With Photo-Electric Oxyhemoglobinograph, Ann. Surg. **112:** 309-312, 1940.
17. Goldie, E. A. G.: A Device for the Continuous Indication of Oxygen Saturation of Circulating Blood in Man, J. Scient. Instruments **19:** 23-25, 1942.
18. Wood, E. H., and Geraci, J. E.: Photoelectric Determination of Arterial Oxygen Saturation in Man, J. LAB. & CLIN. MED. **34:** 387-401, 1949.
19. Wood, E. H.: A Single Scale Absolute Reading Ear Oximeter, Proc. Staff Meet. Mayo Clin. **25:** 384-391, 1950.
20. Wood, E. H.: The Oximeter, in Otto Glasser's: Medical Physics, ed. 2, Chicago, 1950, The Year Book Publishers.
21. Elam, J. O., Neville, J. F., Sleator, W., and Elam, W. N.: Sources of Error in Oximetry, Ann. Surg. **130:** 755-773, 1949.
22. Sleator, W., Elam, J. O., Elam, W. N., and White, H. L.: Oximetric Determinations of Cardiac Output Responses to Light Exercise, J. Applied Physiol. **3:** 649-664, 1951.
23. Paul, W.: Contribution to Oximeter Design Using Modulated Light, Fed. Proc. **9:** 99, 1950.
24. Comroe, J. H., and Wood, E. H.: Measurement of O_2 Saturation by Filter Photometers (Oximeters), In Methods in Medical Research, Vol. 2, J. H. Comroe, Editor-in-Chief, Chicago, 1950, The Year Book Publishers.
25. McClure, Roy D., Behrmann, V. G., and Hartman, F. W.: The Control of Anoxemia During Surgical Anesthesia With the Aid of the Oxyhemograph, Ann. Surg. **128:** 685-707, 1948.
26. Burchell, H. B.: An Introduction to the Clinical Applications of Oximetry, Proc. Staff Meet. Mayo Clin. **25:** 377-391, 1950.
27. Personal Communication from Mr. J. Busser, formerly of The Johnson Foundation, University of Pennsylvania, Philadelphia, Pa., who assisted the late Dr. G. A. Millikan in this project.
28. Hemingway, A., and Taylor, C. B.: Laboratory Tests of the Oximeter with Automatic Compensation for Vasomotor Changes, J. LAB. & CLIN. MED. **29:** 987-991, 1944.
29. Liston, M. D., Quinn, C. E., Sargeant, W. E., and Scott, G. G.: A Contact Modulated Amplifier to Replace Sensitive Suspension Galvanometers, Rev. Scient. Instruments **17:** 194-198, 1946.
30. Hartman, F. W., Behrmann, V. G., and Chapman, F. W.: A Photo-Electric Oxyhemograph, Am. J. Clin. Path. **18:** 1-13, 1948.
31. Behrmann, V. G., and Hartman, F. W.: The Value of Continuous Arterial Oxygen Saturation Recordings in Anesthesia, J. Am. Assoc. Nurse Anesth. **16:** 182-190, 1948.
32. Hartman, F. E., Behrmann, V. G., and McClure, R. D.: The Oxyhemograph, Am. J. Surg. **78:** 867-869, 1949.
33. Wood, E. H.: Personal communication, 1950.

LABORATORY METHODS

A DILUTING FLUID FOR COUNTING ERYTHROCYTES WHICH SIMULTANEOUSLY STAINS THE CELLS AND MAKES THEM LIE FLAT WITHIN A SINGLE FOCAL PLANE

OVERDO T. GEORGE*
TORONTO, ONTARIO

IN 1922, Waugh[1] described a technique which employed eosin for staining erythrocytes to facilitate their enumeration, but his method differed in almost all other respects from those commonly employed such as described by Kolmer and Boerner.[2] In this laboratory, the possibility has been explored of staining red cells by incorporating eosin in a conventional diluting fluid. The method developed not only successfully imparts a brilliant red to the cells to be counted, but also exhibits an unexpected phenomenon greatly facilitating the speed and accuracy of the counts. In the new fluid, the blood cells lie flat and are evenly distributed within a single focal plane in the counting chamber. Use of the eosin-containing fluid has now been tested on peripheral bloods obtained from a large number of human patients and from experimental animals. It has proved to give consistent results.

METHODS AND MATERIALS

The new counting fluid is a modification of a conventional one described by Dacie[3] with eosin added as the coloring agent. Various concentrations of this dye were tried but, to avoid undesirable effects such as crenation or hemolysis, it was found that 0.6 Gm. per 100 ml. was optimal. The composition of the diluting fluid as finally adopted was:

3 per cent aqueous sodium citrate	100.0 ml.
Formalin (commercial, 40 per cent)	1.0 ml.
Water-soluble, bluish eosin†	0.6 Gm.

After it had been filtered, the mixture was ready for use and kept indefinitely. Difficulty in cleansing pipettes has not been encountered in this laboratory, although it was reported by others. This was overcome by first rinsing the pipettes in ammoniated water.

A large number of red blood cell counts have been made with the eosin-containing diluting fluid on samples of peripheral bloods from men and women, as well as from various laboratory animals including rats, guinea pigs, rabbits, cats, and dogs. Collection of the bloods, dilution, mechanical mixing, and actual counting of the erythrocytes in a Neubauer-type hemacytometer were done by the usual routine procedures. In many cases, comparative counts were made in which dilutions of the same bloods were prepared with both the new fluid and a conventional one.

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*Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.
†Eosin, color index 348, obtained from Messrs. G. T. Gurr, London, England.

TABLE I. ACTUAL COUNTS FOR NORMAL BLOODS
(MILLIONS OF CELLS PER CU. MM.)

	HUMAN	HUMAN	DOG	DOG	RAT
Counts with eosin-containing fluid	3.71	4.80	5.83	4.77	6.32
	3.49	4.72	5.78	4.55	6.45
	3.71	4.60	5.44	4.45	6.15
	3.45	4.63	5.59	4.60	6.40
	3.60	4.77	5.87	4.58	6.30
Averages	3.57	4.70	5.70	4.59	6.32
Counts with regular fluid	3.10	4.65	5.38	4.70	6.13
	3.52	4.53	5.23	4.36	6.21
	3.02	4.58	5.10	4.64	6.15
	3.50	4.50	5.21	4.50	6.20
	3.55	4.69	5.07	4.45	6.00
Averages	3.36	4.59	5.19	4.53	6.14

RESULTS

The actual results obtained when repeated counts were made on each of five normal bloods with both the conventional diluting fluid and the new, eosin-containing one are compared in Table I. The average of five counts made with each diluting fluid for ten pathologic bloods are presented in Table II. In almost every case, the range of counts with the new fluid is similar to the range of those obtained with the conventional one; however, the actual values are significantly ($p < 0.001$) higher with the former. This difference was apparent even with samples of blood from patients with hemolytic anemia which exhibited increased erythrocyte fragility and spherocytosis. There was no evidence of hemolysis when these bloods were diluted with the new fluid, and the spherocytes were still readily distinguishable. Another significant observation was that, in the eosin-containing fluid, all the cells lay flat and were more uniformly distributed in the counting chamber. None remained on its edge, as frequently occurred with the conventional diluent. This is illustrated by Figs. 1 and 2.

DISCUSSION

It is surprising that counts with the conventional diluting fluid apparently failed to demonstrate as many cells as did those with the new eosin-containing fluid. It would seem that if red cells are counted in the unstained condition, some of them lack sufficiently high refractive indices to be readily visualized,

TABLE II. AVERAGE COUNTS FOR ABNORMAL BLOODS
(MILLIONS OF CELLS PER CU. MM.)

CLINICAL DIAGNOSIS	DILUTING FLUID	
	EOSIN-CONTAINING	CONVENTIONAL
Idiopathic hypochromic anemia	3.29	3.11
Hypochromic microcytic anemia	4.95	4.72
Hypochromic microcytic anemia	1.64	1.60
Pernicious anemia	1.56	1.47
Pernicious anemia	2.63	2.64
Hemolytic anemia	4.77	4.60
High hemoglobin	4.48	4.24
Lymphoma	1.70	1.63
Lymphatic leukemia	2.29	2.15
Myelogenous leukemia	1.85	1.82

a suggestion which gains support from observations made with the phase-contrast microscope. This might be related to the age of the red cells, but we have no evidence to support such an hypothesis. In fact, even with the eosin-containing fluid some cells are less prominent than others, and it is possible that similar ones would be missed entirely if suspended in the usual types of diluting fluids. If the error resulting from such a source were always constant and

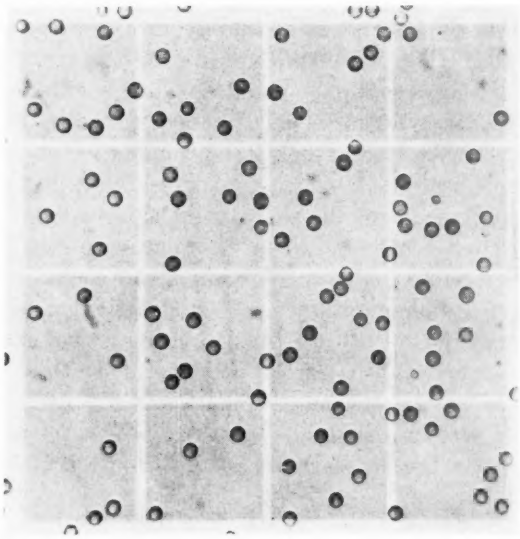

Fig. 1.—Cells suspended in the new diluent, which employs eosin as a coloring agent. Note that every cell lies flat and simultaneously in focus.

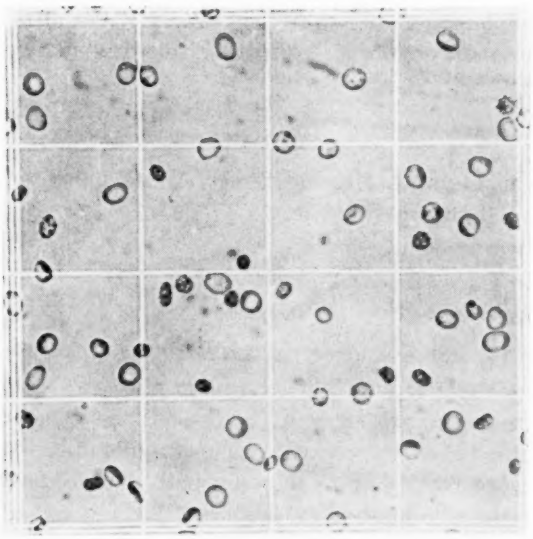

Fig. 2.—Cells suspended in standard diluent. Note that some cells are on edge and some are almost out of the focal plane.

reproducible, it would not seriously interfere with comparative counts, but the possibility remains that, under some pathologic condition, the proportions of "pale" cells might vary, thus leading to consequent variations in this source of error. There is no guarantee, of course, that even the new fluid renders all erythrocytes visible, indeed, phase-contrast microscopic studies suggest quite the opposite. It is clear from the tables, however, that the new fluid reduces possible errors due to not readily visualized cells.

It is remarkable that the stained cells lie so uniformly and evenly within the counting chamber. We are unable to account for this phenomenon, which may also help to explain the higher counts obtained with the eosin solution—the thickness of erythrocytes is so small compared with their diameters that cells lying on edge may appear much less prominent than when they lie flat. It is certainly easier to decide whether a given cell on a line should be included within any particular square of the counting chamber when it is lying flat than when it is tipped on edge over the line. Elimination of the necessity for readjustments of focus increases the speed with which counts may be made, and the observer experiences less fatigue and eyestrain when doing a number of counts at a single sitting. With all cells so clearly visualized in the plane of their greatest diameters, it is scarcely possible to confuse them with debris, for most frequently the latter has been mistaken for cells on edge. All the advantages of the new counting fluid, when used with human blood, are present also when it is used in the enumeration of erythrocytes of common laboratory animals.

The cells appear red against a pink background. Attempts have been made to devise a fluid which would provide a greater color contrast between cells and background. Polychromatic dyes, such as methylene blue, gentian violet, and crystal violet, were tried in the hope that the cells would stain one color and the fluid another; but the cells were very poorly visualized. It is possible that with further investigation, a dye may be found which will provide greater color contrast than does eosin.

SUMMARY

A new, eosin-containing diluting fluid for use in enumerating red blood cells is described. Its composition is:

3 per cent aqueous sodium citrate	100.0 ml.
Formalin (commercial, 40 per cent)	1.0 ml.
Water-soluble, bluish eosin	0.6 Gm.

The mixture is ready for use after being filtered, and keeps indefinitely.

The advantages claimed for this fluid are that (a) the contrast between cells and background is improved, and (b) the cells lie flat within a single focal plane in the counting chamber. The relationship of the cells to the rulings can thus be decided more readily and confusion with debris is minimized. Clearer visualization and the elimination of the need for focal readjustments of the microscope while counts are being made considerably increase the speed with which they can be completed and reduce eyestrain.

The new, eosin-containing diluting fluid has given consistent and reproducible results not only with normal and pathologic bloods from human beings, but also with blood from a number of common laboratory animals including rats, guinea pigs, rabbits, cats, and dogs.

Higher red blood cell counts are usually obtained with the new fluid. Possible reasons for these and their significance have been discussed.

The author gratefully acknowledges the suggestions and assistance provided by Drs. W. Stanley Hartroft and W. G. Bruce Casselman. Mrs. Phyllis Merritt Hartroft assisted with the statistical evaluation of the results. The samples of pathologic and some normal human bloods were provided by the laboratories of hematology of the Toronto General Hospital and the Hospital for Sick Children, Toronto, Canada.

REFERENCES

1. Waugh, T. R.: An Efficient and Practical Method for Counting of Red Cells, Arch. Int. Med. 30: 216-220, 1922.
2. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technic, New York, 1941, D. Appleton-Century Company, Inc., p. 68.
3. Dacie, J. V.: Practical Haematology, London, 1950, J. & A. Churchill, Ltd., p. 155.

A NEUROSURGICAL AND MULTIPLE PURPOSE MONKEY CHAIR

LT. JAMES E. ZIEGLER, MEDICAL CORPS, UNITED STATES NAVY
JOHNSVILLE, PA.

THE demand for a reasonably comfortable restraining device which would stabilize a monkey's head during craniotomy under local anesthesia led to the design and construction of the neurosurgical and multiple purpose monkey chair, in 1947, at the Montreal Neurological Institute, Montreal, Canada.[1]

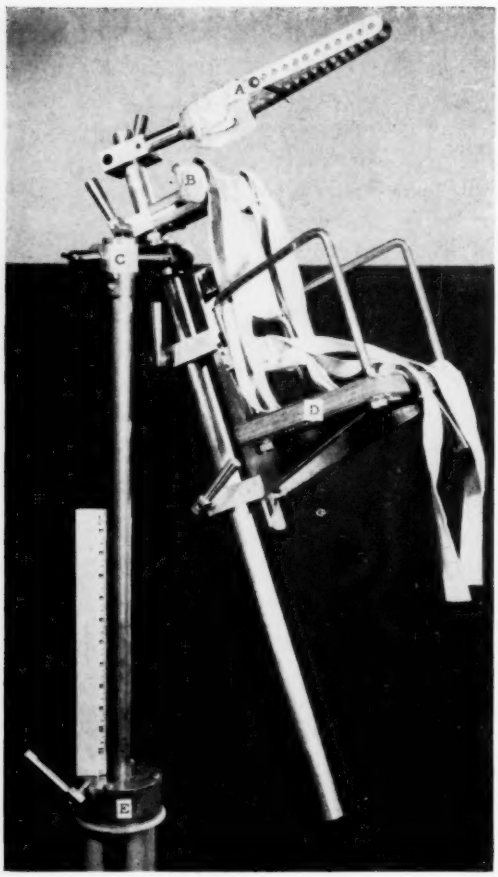

Fig. 1.

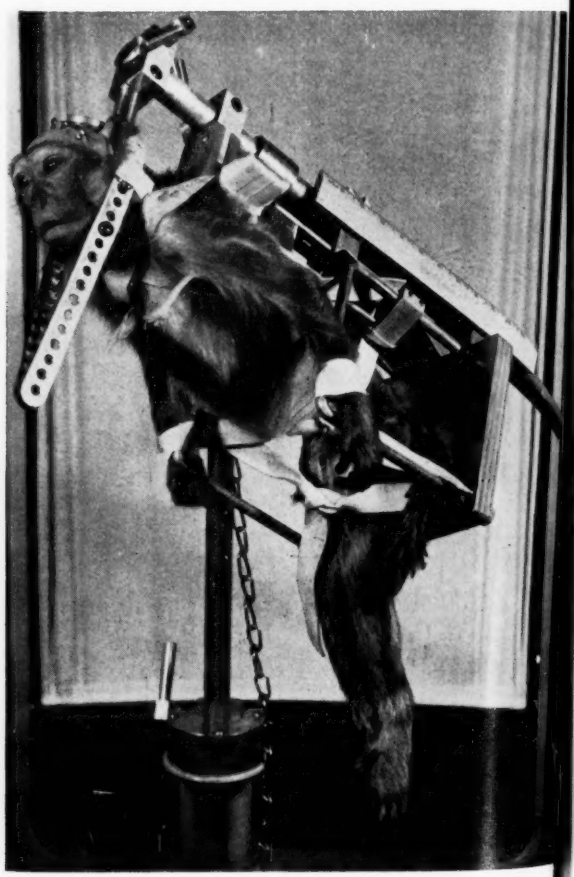

Fig. 2.

Fig. 1—Ziegler neurosurgical and multiple purpose monkey chair. *A*, Head-holder; *B*, shoulder rest; *C*, modified universal joint; *D*, chair seat; *E*, chair base.

Fig. 2.—Monkey with window in position to hold saline while window is inserted—preventing air bubbles.

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Opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department. Reference may be made to this report in the same way as to any published articles; noting author, title, source, and date.

DESCRIPTION

A. Chair.—The chair is composed of three components which slide on a tube, thus adjusting to any size monkey (Fig. 1). These components include the head holder, shoulder rest, and seat. In order to tighten the head in the holder, the seat is slipped down the tube, which wedges the monkey's jaw and the sides of his head in the holder. The shoulder rest is then slipped down the tube which further immobilizes the monkey (Fig. 2). The arms are strapped to the arm rest with adhesive tape. If the monkey is comfortable, he struggles very little.

B. Universal Joint.—This allows positioning of the chair in any desired position (Fig. 1, *C*).

C. Base.—The base is filled with lead shot in order to prevent tipping. The entire chair may be raised or lowered above the base on a tube which clamps into the base (Fig. 1, *E*).

USE

Present uses of the chair include craniotomy under local anesthesia with stimulation and recording from the exposed cortex, implantation of cranial windows, general restraint for dressings anesthesia, and as a seat for the monkey in various positions on the large experimental centrifuge.

DISCUSSION AND SUMMARY

The chair is adjustable to any size monkey and is both washable and durable. The head and extremities and large areas of the body are exposed and thus accessible for various manipulations (Fig. 2). By means of a locking universal joint (Fig. 1, *C*), the monkey may be positioned head up or head down, supine or prone, and on either his right or left side; as well as any combination of these positions. For sterile operations the entire monkey and chair are draped so that positioning may be carried out by the operator without breech of the sterile technique.

REFERENCE

1. Ziegler, James E.: Methods for Temporary and Reversible Paralysis of Local Areas of the Cerebral Cortex (Thesis), Presented to the Faculty of Graduate Studies and Research of McGill University in Partial Fulfillment of the Requirements for the Degree of Master of Science: 56-57, 1949.

APPARATUS FOR TUBE-FEEDING RATS

P. Talalay, M.D.,* and G. M. Y. Takano, B.A.
Chicago, Ill.

CONTROLLED dietary intake of experimental animals by gastric intubation affords a means of: (1) maintaining adequate dietary intake in animals who have lost appetite; (2) assuring accurately known dietary intake for each individual animal; (3) producing persistently overfed animals; (4) introducing unpalatable diets; or (5) administering known doses of drugs. The principal objection to this procedure is that it is time consuming and tedious. After a little experience, passing a catheter into the stomach can be accomplished rapidly and without trauma. The time-consuming element is in handling and quantitatively dispensing the rather viscous diet.

During the course of a quantitative study of the growth of transplantable rat tumors, it became necessary to tube feed large numbers of rats, at eight-hour intervals for many days. The automatic diet-dispensing machine described herein has effected a considerable saving in time.

CONSTRUCTION OF APPARATUS

The machine constitutes an automatic volumetric disepnser for highly viscous liquids. The cardinal features and constructional details are shown schematically and in the photograph (Fig. 1). An air cylinder (*A*) (4″ stroke by 1″ bore), fitted with ⅛″ female pipe inlets, is connected by means of a brass coupling (*B*) to the plunger of a Sani-Lok Control Syringe (*C*) graduated to 10 ml. (capacity about 13 ml.). The thumb ring has been removed from the metal plunger. In this type of syringe the short metal piston sliding within a glass barrel tends to minimize frictional forces. The syringe outlet is coupled by Luer-Lok to a catheter adapter from which a short length of nonexpansible plastic tube leads to two one-way valves (*D*) mounted vertically.†

The lower valve is fitted with a rubber tip which dips into the plastic food container (*E*). The diet is stored frozen in such plastic containers and warmed to body temperature by running hot water over the sealed container just prior to use. The upper valve (*F*) leads, by a flexible, nonexpansible connection, to a Guyon catheter adapter to which a No. 8 French soft rubber catheter‡ is securely attached.

The excursion of the piston (and hence the volume of liquid measured and ejected) is governed by means of a screw adjustment (*L*) which limits the extent of the return stroke of the cylinder. The extent of the forward or feeding stroke is so adjusted that the syringe is always completely emptied.

The air cylinder is activated by compressed air (20 to 30 pounds per square inch) which enters at *G*. All pipe fittings are ⅛″ and interconnections are made of ¼″ (outside diameter) copper tubing and compression valve fittings. The compressed air is distributed via a "T" to two Skinner stainless steel, three-way solenoid valves§ (H_1) and (H_2). The valves are so connected that without energizing their coils, valve (H_1) is normally open and valve (H_2) is normally closed to the passage of compressed air, whereas valve exhaust (X_1) is closed and (X_2) is open.

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*Senior Assistant Surgeon, Federal Security Agency, United States Public Health Service.

†The all-glass double valve assembly is obtainable from Macalaster Bicknell and Co., Cambridge, Mass.

‡Davol Rubber Company, Providence, R. I.

§Manufactured by the Skinner Chuck Company, Norwalk, Conn.

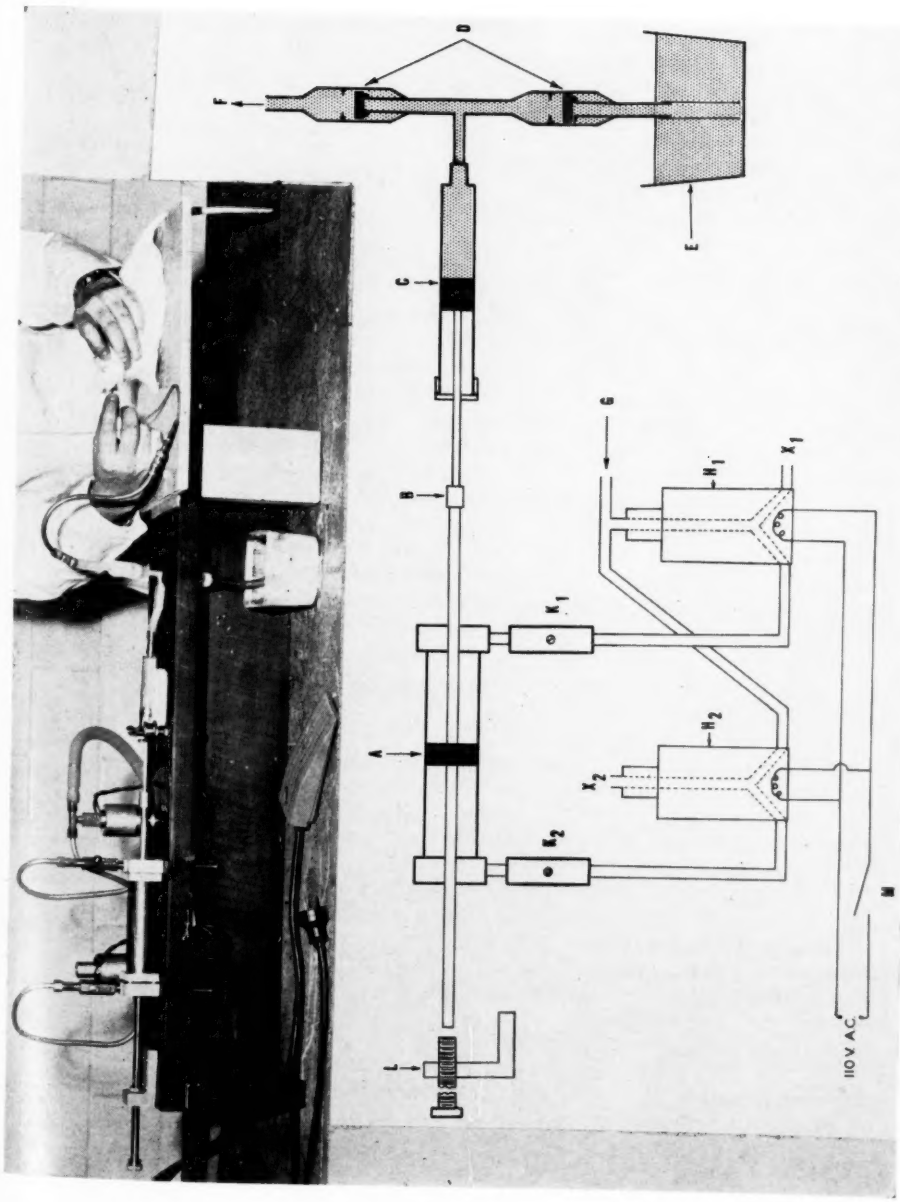

Fig. 1.—Photograph and plan of apparatus for tube-feeding rats. *(For description see text.)*

The two solenoid valves (H_1) and (H_2) are connected in parallel to 110-volt alternating current. On closing the foot-switch (M) the solenoid valve (H_2) opens to the high pressure air and the solenoid (H_1) opens to exhaust the air in the cylinder. The piston is therefore driven in the forward direction to effect feeding. On releasing the foot pedal, the piston travels in the reverse direction and the syringe is refilled with fresh diet. The machine normally rests in the "filling" position. Each solenoid valve is connected through an adjustable flow control valve (Pneu-trol) to its respective side of the air cylinder. These valves* permit control of air flow entering the cylinder and free exhausting of air in the reverse direction. The flow control valves are so adjusted that the feeding stroke is slow and the filling cycle rapid. A convenient, safe rate for feeding 10 ml. of diet may be five seconds, whereas the filling can be accomplished in about two seconds.

If expansible tubing is used in the construction of the machine, there will tend to be dripping from the tip of the catheter at the end of the feeding stroke. This can be avoided by using cord-lined rubber for all flexible connections.

METHOD OF FEEDING

The catheter is readily passed into the stomach with the left hand, by a modification of the technique described by Shay and Gruenstein.[1] Immobilizing the rat, yet affording it the maximum degree of comfort possible, is essential in the tube-feeding procedure. The animal in the natural prone position, but with the hind legs extended, is held with the right hand such that the middle and ring fingers firmly lock around the upper right foreleg and continue around the chest to meet the thumb which is held against the left side of the head, and in this manner a loose ring is formed. Forward movement of the rat is thus restricted without the danger of compressing the chest. The right forefinger and thumb form a vise to immobilize the lateral movements of the head and jaw. The right forefinger holds the jaw open and simultaneously extends the head and neck. The palm of the hand immobilizes the hind part by pressing the extended animal against the table.

Diets containing soluble albumin or casein as protein source may be successfully fed with this apparatus. For maintenance of good nutrition in rats, we have routinely used a diet consisting of (per cent by weight): 23.4 Dextrin, 1.9 corn oil, 2.5 Ruffex, 11.1 soluble albumin (85 per cent protein nitrogen), 2.0 Hawkins-Oser modification of Osborn-Mendel salt mixture, 0.5 vitamin B mixture, 58.6 water, plus 0.3 drop oleum percomorphum and 5 mg. synthetic vitamin K per 100 Gm. of diet. About 24 ml. of this diet is required daily by a 200 to 250 Gm. rat for normal weight gain. We prefer to feed 8 ml. three times each day at eight-hour intervals. Hypophysectomized, adrenalectomized, and intact rats may be maintained in excellent condition by such a regime.

Intact animals tolerate 12 ml. of diet twice daily, but for hypophysectomized rats three daily feedings of 8 ml. each are essential.

The animals are conditioned to take the full diet by feeding successively increasing amounts. For example, hypophysectomized animals are fed 2 ml. three times daily on the first day and the size of the feedings is increased by 1 ml. daily until the full measure of 24 ml. is attained in seven days.

Once the animals are conditioned, one person can feed one hundred 250 Gm. rats 8 to 12 ml. of diet in thirty minutes.

SUMMARY

The construction of a machine for tube-feeding rats with a highly viscous diet has been described. This apparatus permits quantitative dispensing of diet with ease and rapidity to large numbers of animals.

The authors gratefully acknowledge the helpful suggestions of Dr. Dwight J. Ingle and the technical advice of Mr. Leon Talalay.

REFERENCE

1. Shay, H., and Gruenstein, M.: A Simple and Safe Method for the Gastric Instillation of Fluids in the Rat, J. LAB. & CLIN. MED. 31: 1384-1386, 1946.

*Obtainable from Pneu-trol Devices, Inc., Chicago, Ill.